Transplant Immunology 29 (2013) 22–27

Contents lists available at ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

Peri-operative third party red blood cell transfusion in renal transplantation and the risk of antibody-mediated rejection and graft loss☆

Samantha Fidler [a,b,1], Ramyasuda Swaminathan [c,d,2], Wai Lim [e,3], Paolo Ferrari [f,4], Campbell Witt [a,b,5], Frank T. Christiansen [a,b,6], Lloyd J. D'Orsogna [a,b,7], Ashley B. Irish [c,d,*,8]

[a] Department of Clinical Immunology, PathWest, Royal Perth Hospital, Western Australia, Australia
[b] School of Pathology and Laboratory Medicine, University of Western Australia, Australia
[c] Medical Renal Transplant Unit, Royal Perth Hospital, Western Australia, Australia
[d] School of Medicine and Pharmacology, University of Western Australia, Australia
[e] Department of Nephrology Sir Charles Gairdner Hospital, Perth, Western Australia, Australia
[f] Department of Nephrology Fremantle Hospital, Fremantle, Western Australia, Australia

ARTICLE INFO

Article history:
Received 12 August 2013
Received in revised form 23 September 2013
Accepted 23 September 2013

Keywords:
Donor-specific antibody (DSA)
Peri-operative transfusion
Kidney transplantation
Antibody mediated rejection

ABSTRACT

Historic red blood cell transfusion (RBCT) may induce anti-HLA antibody which, if donor specific (DSA), is associated with increased antibody-mediated rejection (AMR). Whether post-operative RBCT influences this risk is unknown. We examined the RBCT history in 258 renal transplant recipients stratified according to prevalent recipient HLA antibody (DSA, Non-DSA or No Antibody).

AMR occurred more frequently in patients who received RBCT both pre and post transplant compared with all other groups (Pre + Post-RBCT 21%, Pre-RBCT 4%, Post-RBCT 6%, No-RBCT 6%, HR 4.1 $p = 0.004$). In the 63 patients who received Pre + Post-RBCT, 65% (13/20) with DSA developed AMR compared with 0/6 in the Non-DSA group and 2/37 (5%) in the No-Antibody group (HR 13.9 $p < 0.001$). In patients who received No-RBCT, Pre-RBCT or Post-RBCT there was no difference in AMR between patients with DSA, Non-DSA or No-Antibody. Graft loss was independently associated with Pre + Post-RBCT (HR 6.5, $p = 0.001$) AMR (HR 23.9 $p < 0.001$) and Non-AMR (6.0 $p = 0.003$) after adjusting for DSA and delayed graft function.

Re-exposure to RBCT at the time of transplant is associated with increased AMR only in patients with preformed DSA, suggesting that RBCT provides additional allostimulation. Patients receiving Pre + Post-RBCT also had an increased risk of graft loss independently of AMR or DSA. Both pre and post procedural RBCT in renal transplantation is associated with modification of immunological risk and warrants additional study.

1. Introduction

The presence of pre-transplant anti-HLA antibody directed against the donor antigens (DSA) in the presence of a negative CDC crossmatch is associated with increased risk of antibody mediated rejection (AMR) and graft failure [1–3]. HLA antibodies are formed as a consequence of prior transplantation, pregnancy and blood transfusion due to exposure to foreign HLA antigens [4–9]. However blood transfusion prior to transplant is immunomodulatory and appears to reduce the risk of acute allograft rejection and graft loss despite an increased risk of sensitisation [10–12]. Historically it had been observed that large volumes of third-party red blood cell transfusion (RBCT) (up to 20 units) over a prolonged period are required to induce enduring antibodies, especially in males or nulliparous females [4,13–15]. However in the presence of another immune stimulating process such as pregnancy or transplantation, co-administration of third party RBCT results in broad HLA antibody production which is more potent and enduring [6,16,17]. In the

Abbreviations: AMR, antibody-mediated rejection; RBCT, red blood cell transfusion; cPRA, calculated panel reactive antibody; DSA, donor specific antibodies; DGF, delayed graft function; BPAR, biopsy proven acute rejection; MFI, mean fluorescence intensity; SAB, single antigen bead.

* Corresponding author at: Medical Renal Transplant Unit, Royal Perth Hospital, GPO Box X2213, Perth, Western Australia 6847, Australia. Tel.: +61 8 9224 2162; fax: +61 8 9224 2160.
E-mail address: Ashley.Irish@health.wa.gov.au (A.B. Irish).

[1] Participated in the performance of research laboratory methods, research design and writing the paper.
[2] Participated with data collection and analysis.
[3] Participated with data collection and writing the paper.
[4] Participated with data collection and writing the paper.
[5] Participated in research design, data analysis and writing the paper.
[6] Participated in research design and writing the paper.
[7] Participated in data analysis and writing the paper.
[8] Participated in research design, data collection and analysis and writing the paper.

0966-3274/$ – see front matter. Crown Copyright © 2013 Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.trim.2013.09.008

current transplant era, transfusion in patients with end stage kidney disease is less frequent due to the widespread use of epoetins. However during acute illness or surgery patients may still be exposed to blood products, although specifically transfusing patients for immunological benefit is no longer routine [18–20]. Leucodepletion of blood products has also been shown not to prevent the risk of allosensitisation associated with RBCT [14,21–23]. The majority of studies on the role of blood transfusion was performed in the period before the use of sensitive and specific solid phase antibody detection assays were available and cell-dependent cytotoxicity assays were utilised. Although it is established that DSA detected at the time of transplant is associated with an increased risk of AMR why some patients with DSA develop AMR and others do not is unclear and may relate to variability in the antibody sub-type, complement binding ability, or the amount or breadth of antibody [1,24–26]. Transfusion in the peri-operative and early post-transplant period depends on individualised patient management factors and is commonly thought not to be an immunological stimulus because it is assumed that the concomitant use of immunosuppression mitigates this risk. We hypothesised that post-transplant transfusion in patients with preformed HLA antibody may provide additional allostimulation or immunological recall and increase the risk of AMR. We therefore investigated the relationship of pre-transplant and peri-operative transfusion in renal transplant recipients with and without pre-transplant HLA antibody determined by Luminex single antigen bead (SAB) assay.

2. Subject and methods

2.1. Patients

We studied 258 transplant recipients of which 246 patients received a kidney transplant and 12 patients received a simultaneous pancreas–kidney transplant between June 2003 and October 2007. Patients were transplanted at 3 tertiary centres and peri-operative care and decision for transfusions was individualised, clinically indicated and not mandated by protocol. No donor-specific transfusions occurred. Leucocyte depleted packed red cells were used. All patients received a calcineurin inhibitor (CNI) (tacrolimus or cyclosporine) at the time of transplantation in combination with mycophenolate mofetil or mycophenolate sodium and corticosteroids and the Interleukin-2 receptor antibody basiliximab was commonly used for induction. The need for biopsy, medication adjustments and transfusion was determined by the caring clinical teams and was not protocol driven. Transfusion history was obtained from the West Australian Red Cross Blood Bank, the Westmead Hospital Transfusion Laboratory, patient medical records and direct patient interrogation. Patient follow-up was a median of 67 months (IQR 54–77). Patients provided written consent for participation in this study.

2.2. Laboratory methods

These are reported in detail elsewhere however stored donor DNA was typed by sequence based typing at HLA-A, -B, -C, -DRB1, DQB1, DPB1 loci and DRB3, 4, 5 and DQA1 where required [27]. All recipients were transplanted with a negative T cell CDC crossmatch. B cell crossmatching was performed for 80% of the patients; however a positive B cell crossmatch was not considered an absolute contraindication to transplantation. Sera collected at the time of transplant were screened retrospectively for anti-HLA class I and/or class II antibodies using the Luminex Mixed Screen assay (OneLambda Inc.) and those with a positive screen were characterised for HLA class I and/or class II antibodies specificity using single antigen beads (LABScreen Single Antigen beads, OneLambda Inc.). Antibodies were considered to be positive if the normalised mean fluorescence intensity (MFI) value for a particular bead was greater than 500. HLA antibodies with an MFI >500 directed against a donor HLA antigen were considered to be DSA.

2.3. Clinical outcome parameters

Transfusion history was recorded as never transfused (No-RBCT), transfused at any time prior to renal transplant but not after renal transplant surgery (Pre-RBCT), not transfused prior to transplant but transfused at the time of, or within 30 days of transplant surgery (Post-RBCT) and transfused both prior to and within 30 days of the transplant (Pre + Post-RBCT). Delayed graft function (DGF) was defined as the need for dialysis within the first 48 h of transplantation. Graft loss was defined as the return to dialysis (i.e. death-censored) unless otherwise indicated. All rejection episodes were proven by biopsy (BPAR) and the first BPAR was used to construct time to event analysis and where multiple rejections occurred, the highest reported grade was recorded. Time to AMR was recorded as a separate event to allow analysis by rejection type (AMR vs Non-AMR). Treatment of rejection was at the treating clinician's discretion and was not mandated by protocol. Histological reporting of renal biopsies was undertaken by the local histopathologists as part of routine clinical care and was initially made without information as to the presence or absence of DSA (due to varying laboratory testing and reporting changes over the period of study). The biopsy findings were graded according to the Banff classification 2003. AMR was defined as C4d positivity in PTC alone or in conjunction with transplant glomerulitis and/or peri-tubular capillaritis and/or arteritis, and also in the absence of C4d when transplant glomerulitis and peri-tubular capillaritis were detected.

2.4. Statistical analysis

Statistical analyses were performed by using SPSSv18 (SPSS Inc., Chicago IL, USA). For categorical data Fisher's exact test or Pearson's chi-square tests were used. Parametric data were compared by ANOVA or *t*-test, and for non parametric data Mann–Whitney *U* test or Kruskal–Wallis one-way ANOVA was used. Comparisons of within group differences by z-test were made with Bonferroni adjustment reported at the $p < 0.05$ level. Time to event of interest (AMR, graft and patient survival) was estimated by the method of Kaplan–Meier and Cox proportional hazard regression analysis with the predictor satisfying the proportional hazard assumption. Covariates examined were HLA-antibody at entry, rejection, gender, re-transplantation, and delayed graft function. Results were expressed as hazard ratios (HR) with 95% CI.

3. Results

Sixty-five patients had pre-transplant HLA-antibody: DSA group n = 37 (14%) and Non-DSA group n = 28 (11%) while the remaining 193 (75%) patients had no HLA antibody defined using the MFI cut-off of <500 or with a negative antibody screen. Baseline clinical and demographic data of these groups is reported in detail elsewhere and summarised in Table 1. [27]As expected, patients with any HLA antibody were more commonly female (41/65 vs 53/193 $p = 0.003$) and more likely to have undergone prior kidney transplant (20/65 vs 7/193 $p < 0.001$) and to have received Pre-RBCT (39/65 vs 70/193 $P = 0.011$). There was no difference in haemoglobin between the groups either at time of transplant (DSA 124 ± 19, Non-DSA 124 ± 18, No-Antibody 124 ± 15 g/L $p = 0.99$) or at 30 days post transplant (DSA 109 ± 17, Non-DSA 113 ± 13, No-Antibody 114 ± 17 g/L $p = 0.19$). Patients with pre-transplant DSA were significantly more likely to have been transfused within the first 30 peri-operative days (DSA 70%) than those with Non-DSA (43%) or no HLA antibody (38% $p < 0.001$) although the amount of RBCT was not different [DSA 4 (2–4), Non-DSA 2 (2–4) and No-Antibody 2 (2–4) units median and IQR $p = 0.17$] and >90% of all post-transplant RBCT given within the first 2 peri-operative days.

In order to explore further the relationship between transfusion and pre-transplant DSA we divided the patients into four groups according to their transfusion status — No-RBCT, Pre-RBCT, Post-RBCT and Pre + Post-RBCT groups as previously defined (Table 2). Overall 109/258 (42%) received Pre-RBCT and 111/258 (43%) of patients received Post-RBCT. The prevalence of HLA antibody amongst these groups varied significantly as expected. The No-RBCT group were much more likely to have no HLA antibody (86%) than the other groups ($p < 0.05$). Conversely however, the Pre + Post-RBCT group were more likely to have DSA ($p < 0.05$), receive a repeat transplant and less likely to receive a pre-emptive or living donor transplant, although time on dialysis was similar to those with Pre- and Post-RBCT. Patients with Pre-RBCT only were significantly less likely to have Non-AMR rejection than all other groups ($p < 0.05$ Table 3). Patients in the