

Title

A systematic review of the use of rituximab for the treatment of antibody-mediated renal transplant rejection

Abstract

Rituximab is a B-lymphocyte depleting agent that is used to treat hematological malignancies and autoimmune diseases. Recently, it has gained interest as an immunomodulatory agent in renal transplantation. This systematic review evaluates the evidence for its use in the treatment of acute and chronic antibody-mediated renal transplant rejection (AAMR; CAMR). A systematic search of four databases and three trial registries was conducted. The small number and heterogeneous nature of included studies precluded meta-analysis and thus a narrative review was conducted. A total of 28 records met the inclusion criteria (AAMR – 18 records relating to 9 studies; CAMR – 10 records relating to 7 studies). Two systematic reviews were identified that had differing inclusion criteria to this current review. Of seven primary studies in the setting of AAMR, four reported increased graft survival and one reported improved graft function with rituximab. This contrasts with CAMR in which only one of seven studies reported improved graft outcomes with a rituximab-based regimen; three studies reported inferior outcomes and three reported no difference. Only one study reported that rituximab was associated with an increase in adverse effects. The included studies suggest that rituximab may be of some benefit in the setting of AAMR but a lack of high quality evidence precludes firm conclusions from being drawn. Rituximab does not appear to reliably improve outcomes in CAMR. Further well-conducted studies are required to better define the effects and long-term safety profile of rituximab in the treatment of antibody-mediated renal transplant rejection.

Key words

Antibody-mediated; rejection; renal; rituximab; systematic; transplantation

Abbreviations

AAMR – acute antibody-mediated rejection

AMR – antibody-mediated rejection

ATG – anti-thymocyte globulin

CAMR – chronic antibody-mediated rejection

CMV – cytomegalovirus

DSAs – donor-specific antibodies

GFR – glomerular filtration rate

ISRCTN – International Standard Randomised Controlled Trial Number

IVIg – intravenous immunoglobulin

MMF – mycophenolate mofetil

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-analyses

PROSPERO – International Prospective Register of Systematic Reviews

RCT – randomized controlled trial

SCr – serum creatinine

Manuscript

Introduction

Rituximab is a chimeric murine/human monoclonal antibody active against CD20, a cell membrane protein found on the surface of B-lymphocytes prior to their terminal differentiation into plasma cells and responsible for regulating their progression through the cell cycle [1]. Rituximab eliminates B-lymphocytes, depleting circulating levels for a period of six to twelve months in 80% patients [2] and thus may help to attenuate antibody-mediated immune responses (although long-lived plasma cells will still persist) [3]. As B-lymphocytes also function as antigen presenting cells, rituximab is also likely to indirectly suppress T-lymphocyte activity [4]. Rituximab has traditionally been used to treat hematological malignancies [5] and autoimmune diseases [6, 7]. More recently it has been used as an immunomodulatory agent in renal transplantation in the settings of induction [8] and desensitization [9] and is also used in the treatment of antibody-mediated rejection (AMR).

Initially, AMR was defined in the revised Banff classification by the presence of histological evidence of rejection along with immunohistochemical demonstration of deposition of the complement component C4d and presence of circulating donor-specific antibodies (DSAs) [10]. However, a recent update following the 2013 Banff meeting also recognizes C4d-negative AMR [11]. Circulating DSAs lead to glomerulitis, inflammation of peritubular capillaries and vascular rejection. AMR occurs most commonly within the first three weeks after transplantation but can develop at any time [12], especially in response to alteration of, or non-compliance with, immunosuppressive therapy.

Acute antibody-mediated rejection (AAMR) occurs in 5-7% of renal transplants [13]; its incidence may be as high as 40% in highly-sensitized recipients [14] but it may also occur in the absence of obvious risk factors. AAMR is a serious event, accounting for 27-40% graft losses within the first year post-transplantation [15]. Unlike acute cellular rejection, AAMR is often resistant to corticosteroids, resulting in a poorer prognosis [16]. Instead, treatment focuses on the removal of DSAs and combinations of plasmapheresis/plasma exchange or immunoadsorption, intravenous immunoglobulin (IVIg), rituximab and alteration of immunosuppressants have allowed 70-80% of affected grafts to be rescued [17]. However, consensus is lacking regarding the optimal management of this condition.

The Banff '05 criteria defined chronic antibody-mediated rejection (CAMR) as a unique entity to avoid the use of the non-specific term 'chronic rejection' [18]. In addition to the presence of circulating DSAs and C4d deposition, biopsies from patients with CAMR display histological features of chronic tissue injury such as transplant glomerulopathy, interstitial fibrosis, tubular atrophy and/or fibrous thickening of the arterial intima. CAMR also has a poor prognosis [19, 20], being one of the leading causes of late graft loss [21], and there is no established treatment.

B-lymphocyte depleting therapies would seem a logical component of treatment for antibody-mediated renal transplant rejection. However, a recent survey of transplant centers in the United States of America regarding the treatment of AMR demonstrated widely varying practices with only 8/28 (28.6%) responding clinicians routinely administering rituximab (an additional two use the drug if first line treatments fail and another two depending upon patient-specific factors) [22]. Therefore, in order to

facilitate treatment decisions, this systematic review aims to critically evaluate all available evidence for the use of rituximab in AMR.

Methods

Protocol and registration

This systematic review was conducted in accordance with the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement [23]. The review is registered with the International Prospective Register of Systematic Reviews (PROSPERO; registration number CRD42012002101).

Eligibility criteria

Inclusion criteria specified all comparative studies of the treatment of antibody-mediated renal transplant rejection (according to Banff criteria [11]) in which rituximab-based protocols were compared with alternative protocols not including rituximab. Exclusion criteria included non-renal solid organ and bone marrow transplant studies, case series and case reports. In order to isolate the effects of rituximab, studies with treatment regimens containing other B-cell depleting agents (including bortezomib) were also excluded. No date or language limits were applied.

Literature search

A systematic literature search was performed in Ovid MEDLINE and Embase, The Cochrane Library and The Transplant Library. Search terms included keywords and free text terms for rituximab and its aliases, along with keywords and free text alternatives for renal transplantation (**Supplementary Material 1**). The final date for searches was 25th July 2016. Reference lists of identified studies were assessed for relevant records not identified by the initial search.

Lead authors of studies reported only in abstract form were contacted for further information and to help identify full publications. Publications identified in this way are included in the analysis. To identify unpublished or in-progress studies, we searched ClinicalTrials.gov (<http://clinicaltrials.gov>), the International Standard Randomised Controlled Trial Number (ISRCTN) Register (<http://www.isrctn.com>) and the World Health Organization International Clinical Trials Registry Platform (<http://apps.who.int/trialsearch>). We also contacted the pharmaceutical companies that manufacture rituximab (Roche [European Union and Canada], Biogen Idec/Genentech [United States of America] and Chugai Pharmaceutical [Japan]).

Study selection

Duplicates were discarded from initial search results. Study selection was performed by two authors (PSM and SRK). The full text of potentially relevant studies was reviewed prior to confirming their inclusion. Any discrepancies that could not be resolved were dealt with by discussion with the third author (PJM).

Data abstraction and analysis

In this review, studies are referred to by the first author and year of their first full publication or published abstract (if no full publication is available). Demographic, outcome and methodological quality data for included studies were abstracted into a database by means of a pro forma. Timing of reported outcomes is recorded as specified in each study; if not specified, average length of patient follow-up is recorded instead. Data is presented as mean (\pm standard deviation) and all results and P values are recorded as reported by each study. The exceptions to this are results that had to be calculated from raw data, which are presented to one decimal place.

Primary study outcomes focus on efficacy including patient and graft survival and graft function. Secondary outcomes focus on safety.

Risk of bias in individual studies

The methodological quality of included studies was evaluated by the Downs and Black Quality Index [24], one of only six tools to evaluate the quality of non-randomized studies deemed appropriate for use in systematic reviews [25]. Quality assessment was performed on the most recent full publication for each study. When studies were reported in abstract form only, quality assessment was not possible.

Synthesis of results

A narrative synthesis was performed because a lack of high-quality randomized studies precluded meta-analysis. Results are presented in a hierarchical order based upon the *Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence* [26].

Results

Study selection and characteristics

28 records met inclusion criteria (11 full articles [27-37] and 17 abstracts [38-54]), relating to 16 distinct studies. Manual searching identified no additional records. Searching trial registries identified 3 other ongoing or unpublished studies that were potentially relevant (ClinicalTrials.gov identifiers NCT00261547, NCT00476164 and NCT00568477). After contacting their lead investigators and the pharmaceutical companies that manufacture rituximab, no further data for inclusion was identified. The flow of trials through the review process is depicted in **Figure 1**.

2 systematic reviews and 1 randomized controlled trial (RCT) were identified; the remaining 13 studies were retrospective cohort studies. 9 primary studies were reported as full papers (and thus were illegible for quality assessment) and 5 were reported in abstract form only. The Downs and Black Quality Index scores for the full publications of primary studies ranged between 19-26/32 (**Supplementary Material 2**). Scoring was stronger in the domains of reporting (whether the manuscript contains sufficient information to permit unbiased assessment of its findings) and external validity (the extent to which the study findings can be generalized to the population from which the study subjects were derived). Weaker scoring was achieved for internal validity (whether the study addresses bias in the selection of study subjects and in the measurement of outcomes) and power (all studies scored 0 for lack of sample size calculation/statistical power).

Acute antibody-mediated rejection

18 records were identified (7 full articles [27-33] and 11 abstracts [38-48]) relating to 9 separate studies that encompass 2 systematic reviews, 1 RCT and 6 retrospective comparative studies (**Table 1**).

Systematic reviews (level one evidence)

Two systematic reviews were identified, both of which included studies that do not meet our inclusion criteria. In a meta-analysis focusing on the use of rituximab as treatment for AMR, Hychko calculated the pooled odds ratio for a response to treatment (at least partial improvement in graft function) as 3.16 (95% confidence interval = 1.75-5.70) [27, 38]. Whilst adverse effects could not be statistically analyzed due to variations in reporting between studies, three comparative studies did not show increased infectious complications with rituximab [30, 55, 56]. However, 8/10 studies are not included in this review, mainly because they do not include a non-rituximab control group. Roberts conducted a narrative systematic review of all treatments for AAMR in kidney transplant recipients [28]. Based on the findings of four non-randomized studies, they concluded that the evidence supporting the use of rituximab was very low. Three studies are included in this review [30, 31, 46] with the remaining study excluded due to concomitant bortezomib administration in the treatment arm [57].

Randomized controlled trials (level two evidence)

In the single identified RCT, Sautenet and colleagues conducted a multi-center, double-blind trial in which 38 patients with AAMR were randomized 1:1 to either rituximab (a single 375 mg/m² dose) or placebo [29, 39-41]. In addition, all patients received plasma

exchange, IVIg and corticosteroids. Depending upon investigators' assessment of clinical response, patients in both groups were eligible for supplementary doses of rituximab (with a maximum of two infusions in total) and additional treatment with plasma exchange and IVIg. In the rituximab group, six patients required an additional infusion whereas eight patients in the placebo group ultimately received rituximab (seven received a single infusion and one received two infusions). Therefore, in the intention-to-treat analysis there were 19 patients in each group whereas in the per protocol analysis there was 27 patients in the rituximab group and 11 in the placebo group.

In the intention-to-treat analysis, there was no difference regarding the trial's primary end point (a composite of graft loss or lack of improvement in graft function at 12 days; 52.6% vs. 57.9%, $P=0.744$). At one year, no deaths occurred but there was one graft loss in each group. Both groups demonstrated significantly improved graft function but there was no difference in graft function between the groups at one, three, six and 12 months. Similar results were seen in the per protocol analysis. Whilst no statistical comparison was provided for safety outcomes, 23 adverse events occurred in the 27 patients who ultimately received rituximab, most importantly opportunistic infections (including BK virus, cytomegalovirus [CMV] and nocardia), gastrointestinal disturbance and one case of malignant melanoma, whilst 14 adverse events occurred amongst the 11 individuals who did not receive the drug (mainly urinary tract infections). The authors conclude that no additional benefit was derived from the administration of rituximab. However, this study suffers from several limitations:

- i. Fewer patients were included than originally planned, reducing the study's power to detect a benefit with rituximab therapy;

- ii. 8/19 patients in the placebo group received supplementary rituximab treatment, potentially masking between group differences (however, all per protocol analyses agreed with intention-to-treat analyses);
- iii. One-year follow-up is too short to detect long-term effects.

Retrospective cohort studies (level four evidence)

Six non-randomized comparative studies were identified, with five reporting positive outcomes with rituximab treatment. In the largest study, Nampoory and colleagues reviewed 103 cases of AAMR and subcategorized them into four groups depending upon the onset of rejection (early vs. late) and the treatment that they received (rituximab [dosage not reported], plasmapheresis and IVIg vs. plasmapheresis and IVIg only) [42-45]. There were no differences in patient survival or adverse effects (development of diabetes mellitus, BK virus infection and malignancy) between the four groups but those treated with rituximab experienced significantly better graft survival ($P=0.028$). From the same institution, a review of 20 cases of pediatric AAMR (ten children with AAMR and ten children with mixed acute rejection) demonstrated that graft survival was significantly higher in those who received rituximab (dosage not reported), plasma exchange and IVIg as compared to other non-specified treatment strategies ($P=0.002$) [47, 48].

Kaposztas reviewed 54 patients treated for AAMR, comparing 26 patients who received plasmapheresis and rituximab (375 mg/m^2 after each plasmapheresis cycle with a mean of 3.61 infusions) to 28 treated with plasmapheresis [30]. IVIg was administered to patients in both groups with low levels of serum immunoglobulin G. Whilst there was no difference in patient survival (96.2% vs. 89.3%), two-year graft survival was greater

amongst patients receiving rituximab (88.5% vs. 60.7%; $P=0.005$). There were no differences in graft function or infectious complications. Whilst significantly more patients in the rituximab group received IVIg as well as a greater number of plasmapheresis treatments, multi-variate analysis suggested that rituximab was the single most important factor behind the difference in graft survival with relative risk of graft loss at two years being five times higher in those not receiving rituximab.

Lefaucher compared the combination of rituximab (two weekly doses of 375 mg/m²), plasmapheresis and IVIg to high-dose IVIg alone in the treatment of biopsy-proven AMR with positive DSAs [31]. 12 patients were included in each group and three-year graft survival was 91.7% in the combination therapy group compared to 50.0% in the IVIg alone group ($P=0.02$). There was no difference in graft function or infectious complications between groups. The improved clinical outcome of the combination therapy group was also mirrored by significantly lower levels of DSAs at three months and this factor was found to be closely associated with the probability of graft survival. The same group also reported that amongst 64 patients suffering from acute rejection with vascular lesions and DSAs, treatment with rituximab, plasma exchange and corticosteroids (22 patients) was associated with a significantly lower risk of graft loss than treatment with corticosteroids and muromonab-CD3 (29 patients) or corticosteroids and IVIg (13 patients; $P\leq 0.03$) [46].

Liu compared eight patients who received rituximab (a single 500 mg dose), anti-thymocyte globulin (ATG) and plasma exchange (two patients) to ten who received ATG [32]. No deaths occurred in either group. Amongst patients treated with rituximab, five experienced an improvement and three a stabilization in renal function. Amongst

the control group, two patients displayed an improvement in renal function whilst eight suffered a deterioration in renal function (six remain on active treatment and two returned to hemodialysis). At both six and 12 months, serum creatinine (SCr) level was significantly lower in the rituximab than the comparison group ($P < 0.05$). Adverse effects amongst the rituximab-treated patients were limited to a single case each of CMV infection and urinary tract infection whilst two pulmonary infections and one urinary tract infection occurred in the control group.

Finally, Steinmetz investigated the use of rituximab in eight cases of acute vascular rejection, comparing outcomes to eight patients with the same diagnosis who did not receive rituximab [33]. The rituximab-treated patients received a single 375 mg/m^2 dose (although one patient received three infusions) and a mixture of corticosteroids, switch from ciclosporin A to tacrolimus, plasmapheresis and IVIg. The comparison group also received variable treatment protocols including corticosteroids, switch to tacrolimus, ATG and IVIg. However, only five patients in the rituximab and six in the comparison group were positive for C4d immunostaining and DSAs measurement was not available. When analyzing data from the C4d positive cases, patient and graft survival were 100% in both treatment groups at three-month follow-up. Mean SCr was broadly comparable between the rituximab and non-rituximab groups, improving from the time of biopsy (2.1 ± 0.4 vs. $2.3 \pm 0.6 \text{ mg/dL}$) to the time of discharge (1.9 ± 0.4 vs. $2.0 \pm 0.5 \text{ mg/dL}$) and at three-month follow-up (1.7 ± 0.6 vs. $1.8 \pm 0.6 \text{ mg/dL}$). No significant adverse effects occurred in either group.

Chronic antibody-mediated rejection

10 records were identified (4 full articles [34-37] and 6 abstracts [49-54]) relating to 7 separate studies, all of which are retrospective comparative studies (**Table 2**).

Retrospective cohort studies (level four evidence)

Only one study reported improved outcomes with rituximab. Chung compared 25 patients with CAMR treated with rituximab (a single 375 mg/m² dose) and IVIg to a group of 29 patients that did not receive these treatments [35, 50]. Significantly more patients in the rituximab group received tacrolimus than the comparison group, most of whom received ciclosporin A ($P<0.01$). A statistically significant increase in graft survival (3 vs. 17 grafts lost; $P=0.03$) and higher glomerular filtration rate (GFR; $P<0.05$) were seen in the rituximab group. There were no serious complications that could be attributed directly to rituximab.

Three studies reported no difference in outcomes with rituximab. In the largest, Redfield described a single institution's experience of managing 123 CAMR patients with a variable combination of rituximab (a single dose of 375 mg/m² or 1000mg), plasma exchange, IVIg, corticosteroids and ATG [34, 49]. Whilst an initial abstract stated that rituximab was the only treatment associated with increased graft survival (hazard ratio of graft loss 0.54 [0.34-0.88]; $P=0.01$) [49], this association was not maintained in the full publication which reported that the addition of rituximab or ATG to corticosteroids and IVIG was associated with a small but statistically non-significant increase in graft survival [34]. However, the reported lack of benefit may in part be explained by selection bias since treatment was based on clinician discretion and thus rituximab may have been more likely to be administered in severe/refractory rejection episodes. In the second, Smith compared the outcomes of 14 patients treated with

rituximab (three to five 375 mg/m² doses) to 17 patients who did not receive the drug [37]. There was one death in the rituximab group and three deaths in the control group. At 2000 days after initial biopsy, graft survival was 28.6% in the rituximab group as compared to 0% in the control group but this fell just short of statistical significance ($P=0.05$). Furthermore, there was no statistical difference in median (685 vs. 439 days) or mean graft survival time between groups. The only adverse outcome associated with rituximab was a case of CMV viremia. Interestingly, eight patients had a significantly better response to rituximab than the remaining six non-responders (median survival 1180 vs. 431 days) but it was not possible to predict in advance which patients would respond. The results of this study must be interpreted in the context of the variable treatment regimens that the subjects received. In addition to rituximab, patients received corticosteroids, mycophenolate mofetil (MMF) and tacrolimus plus a variable combination of plasmapheresis, IVIg, actinomycin and ATG. The control group received MMF plus a variable combination of corticosteroids, ciclosporin A or tacrolimus and IVIg. Ciclosporin A was more commonly used (instead of tacrolimus) amongst control rather than rituximab patients ($P=0.01$), reflecting the historical nature of this group. Finally, Chaparro reported a non-significant trend towards improved graft function in pediatric CAMR when comparing eight patients treated with rituximab (dosage not reported) and IVIg to 24 who received IVIg alone (GFR – 60 vs. 40 mL/minute; $P=0.07$) [52].

The three remaining studies reported poorer outcomes with rituximab. Anwar retrospectively reviewed 39 cases of biopsy-proven CAMR over a ten-year period [51]. Nine patients who received rituximab (a single 200 mg dose) in addition to standard therapy were compared to 30 patients who received standard therapy alone (not

defined). 78% patients suffered graft loss in the rituximab group whereas only 30% lost their graft in the standard therapy group ($P<0.001$). However, these negative findings are tempered by the small number of patients treated with rituximab and the fact that the rituximab group had more advanced disease, with significantly greater renal impairment and proteinuria at presentation. Bachelet compared 21 patients receiving two 375 mg/m² doses of rituximab, four cycles of IVIg and corticosteroids for biopsy-proven transplant glomerulopathy in the setting of CAMR with a historical control group of ten untreated patients [36, 53]. At 24 months, there were no differences in patient survival (95.2% vs. 100%; $P=0.48$), graft survival (47.6% vs. 40%; $P=0.69$) or graft function. At the end of the study, graft survival was also comparable (38.1% vs. 40%; $P=0.99$). However, administration of rituximab was associated with a significant increase in mean number of adverse effects per patient (2.0 vs. 0.5; $P=0.03$); most of these events were infections and cytopenias. Finally, Ghouti-Terki reported that the use of rituximab (dosage not reported), plasma exchange and IVIg in eight patients, compared to 14 patients treated with pulsed corticosteroids, did not lead to improved graft survival ($P=0.27$) [54]. Surprisingly, the patients treated with rituximab and IVIg had a statistically significant decrease in renal function at 12 months (decrease in GFR of 12 mL/minute [$P=0.04$ compared to baseline]) which was not seen in the control group (-6.1 mL/minute [$P=0.72$ compared to baseline]). In addition, rituximab failed to reduce levels of DSAs and there was a progression in the extent of chronic renal damage on biopsy in this group as well.

Discussion

The present systematic review is the most comprehensive analysis of the use of rituximab for the treatment of renal transplant AMR. Although two previous systematic reviews were identified, these used very different inclusion criteria to this current review and neither addressed the efficacy of rituximab in the setting of CAMR. The majority of the studies included in the systematic review by Hychko [27, 38] were not comparative and, as a result, the efficacy of rituximab was assessed by whether or not patients demonstrated improved graft function after treatment. Nevertheless, their findings broadly agree with the results of this study that rituximab may be associated with improved outcomes in AAMR without evidence of an increase in adverse effects. The systematic review by Roberts [28] focused on the efficacy of a wide range of therapies for AAMR and only included a handful of studies relating to rituximab. We agree with their conclusion that the evidence base for the use of rituximab in AMR is weak.

In the treatment of AAMR, five studies reported beneficial effects with rituximab [30-32, 45, 48] and the remaining two studies demonstrated no differences in outcome [29, 33]. Whilst rituximab did not increase patient survival in any study, improvement in graft survival [30, 31, 45, 48] and graft function [32] were reported. Interestingly, positive results were seen in studies in which multiple doses of rituximab were administered [30, 31] whereas no benefit was seen in 2/3 studies in which only a single dose was most commonly given [29, 33]. However, a dose-dependent benefit has not been consistently reported in other studies [58]. In the RCT conducted by Sautenet, the addition of rituximab did not improve upon results seen with plasmapheresis combined

with IVIg. These findings suggest that there is perhaps little benefit in adding rituximab to regimens that are already designed to eliminate alloantibodies.

During literature searching, another RCT potentially relevant to the treatment of AAMR was identified but was omitted from this review, as inclusion was not based on Banff criteria. In this study, Zarkhin and colleagues investigated the use of rituximab in acute renal transplant rejection with B-cell infiltrates in 20 consecutive pediatric patients [56, 59, 60]. Ten patients were randomized to receive standard rejection treatment (corticosteroids with or without ATG) and ten were randomized to standard treatment combined with rituximab. Whilst patient and graft survival did not differ, creatinine clearance was higher in the rituximab group at all follow-up points and biopsy scores were improved in the rituximab but not the standard care group at six months. However, the inclusion criteria for this study were not entirely diagnostic of AAMR; C4d deposition and circulating DSAs were present in only two control patients as compared to six rituximab patients, DSAs were detected in a further two patients in each group without C4d deposition. Therefore, caution must be exercised when extrapolating its results to the use of rituximab for adult patients with classical AMR.

In the setting of CAMR, only one study reported improved outcomes with rituximab [35], three studies reported no difference between treatment strategies [34, 37, 52] and three studies reported poorer outcomes with rituximab [36, 51, 54]. Taken together, these results suggest that rituximab administration may be of some benefit in the acute stages of AMR but is unable to reverse the more advanced tissue damage that characterizes CAMR. Of the studies included in this review, only three compared the extent of tissue injury between intervention groups: Bachelet [36] and Smith [37] found

no difference whereas Ghouti-Terki [54] reported less advanced chronic damage in the rituximab group. Of the four remaining studies, at least two used historic controls [35, 51] meaning that it is less likely (but not impossible) that there was a significant difference in biopsy findings between groups. In studies in which rituximab and comparison patients were treated concurrently, there is a risk that rituximab was given to patients with more severe rejection (for example, in Redfield's study treatment was based on clinician preference [34]) but we feel that this is unlikely to drastically bias our overall conclusion that rituximab lacks efficacy in the treatment of CAMR.

Previous studies have expressed safety concerns regarding the use of rituximab in transplantation, particularly in relation to a theoretical risk of increased infectious complications [61, 62]. However, only one of fourteen primary studies included in this systematic review reported an increase in adverse effects with rituximab [36]. This finding is in keeping with our previous systematic reviews relating to induction [8] and desensitization [9] therapy, neither of which provided convincing evidence of increased adverse effects with rituximab. Whilst conclusive evidence regarding the safety of rituximab in transplantation is awaited, it is reassuring that long-term follow-up of the drug's use in the setting of rheumatoid arthritis has not identified major concerns [63].

This systematic review is limited by a lack of quality evidence and heterogeneity between included studies (particularly in relation to inclusion criteria and treatment protocols) precluded meta-analysis. There is a paucity of RCTs in this field and the two that were identified [29, 56] lack statistical power and long-term follow-up. Instead, the majority of included studies were retrospective and included small numbers of patients, reducing the significance of their findings. Indeed, the apparent benefit of rituximab in

the setting of AAMR may in part reflect poor outcomes amongst historical control groups (for example, in some studies graft survival in the control group was as low as 50-60% [30, 31]). Balancing this effect, it is also possible that rituximab was used as a 'salvage' therapy in unresponsive or more advanced disease. Furthermore, rituximab-treated patients are likely to be subject to shorter follow-up periods than the historical comparison groups, meaning that late adverse effects may be missed. There is also the significant possibility of publication bias in AAMR studies, although it is impossible to formally assess for this.

In addition, the diversity of therapeutic protocols, utilizing a variety of complex medications at differing dosages, means that it is difficult to confidently attribute outcomes solely to the administration of rituximab. Many studies included a variety of components in their treatment protocol other than rituximab, including T-cell therapy, IVIg and plasmapheresis. Whilst some retrospective studies attempted to correct for these differences in analysis, the majority did not. It is likely that the optimum management of AAMR and CAMR will involve a combination of components with different mechanisms of action.

Conclusions

A limited number of published studies suggest that rituximab may have a role in the treatment of AAMR. Therefore, given the current lack of conclusive evidence to support its routine use, it would be more appropriate to view rituximab as a potential 'rescue therapy' in cases of severe AAMR. However, there is no evidence to support a benefit in the setting of CAMR. The lack of high quality evidence precludes firm conclusions from being drawn and this systematic review serves to highlight the need

for high-quality RCTs to better define the therapeutic effect and long-term safety profile of rituximab in the setting of AMR. It is imperative that any such future studies are adequately powered with sufficient follow-up and compare the addition of rituximab to the current standard of care including plasmapheresis and/or IVIg. It will be interesting to see whether the results of the ongoing RituxiCAN-C4 study (NCT00476164) investigating the efficacy of rituximab in CAMR are in concordance with the apparent lack of benefit reported in the studies that we have identified. This study stopped enrolling patients prematurely due to an interim analysis that revealed a smaller than expected effect size and is currently following up recruited patients (personal communication with Professor Anthony Dorling, King's College London).

Acknowledgements

The authors would like to thank all investigators who replied to our requests for additional information about their studies. We would also like to acknowledge Dr Lai Mun Wang from the Department of Cellular Pathology, John Radcliffe Hospital, Oxford, for translating the paper by Liu et al. (2012).

References

1. Tedder, T.F., A. Forsgren, A.W. Boyd, et al., *Antibodies reactive with the B1 molecule inhibit cell cycle progression but not activation of human B lymphocytes*. Eur J Immunol, 1986. **16**(8): p. 881-7.
2. Sureshkumar, K.K., S.M. Hussain, B.J. Carpenter, et al., *Antibody-mediated rejection following renal transplantation*. Expert Opin Pharmacother, 2007. **8**(7): p. 913-21.
3. Clatworthy, M.R., *Targeting B cells and antibody in transplantation*. Am J Transplant, 2011. **11**(7): p. 1359-67.
4. Jordan, S.C., A.A. Vo, D. Tyan, et al., *Current approaches to treatment of antibody-mediated rejection*. Pediatr Transplant, 2005. **9**(3): p. 408-15.
5. Maloney, D.G., A.J. Grillo-Lopez, C.A. White, et al., *IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma*. Blood, 1997. **90**(6): p. 2188-95.
6. De Vita, S., F. Zaja, S. Sacco, et al., *Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis: evidence for a pathogenetic role of B cells*. Arthritis Rheum, 2002. **46**(8): p. 2029-33.
7. Kneitz, C., M. Wilhelm, and H.P. Tony, *Effective B cell depletion with rituximab in the treatment of autoimmune diseases*. Immunobiology, 2002. **206**(5): p. 519-27.
8. Macklin, P.S., P.J. Morris, and S.R. Knight, *A systematic review of the use of rituximab as induction therapy in renal transplantation*. Transplant Rev (Orlando), 2015. **29**(2): p. 103-8.

9. Macklin, P.S., P.J. Morris, and S.R. Knight, *A systematic review of the use of rituximab for desensitization in renal transplantation*. Transplantation, 2014. **98**(8): p. 794-805.
10. Racusen, L.C., R.B. Colvin, K. Solez, et al., *Antibody-mediated rejection criteria - an addition to the Banff 97 classification of renal allograft rejection*. Am J Transplant, 2003. **3**(6): p. 708-14.
11. Haas, M., *An updated Banff schema for diagnosis of antibody-mediated rejection in renal allografts*. Curr Opin Organ Transplant, 2014. **19**(3): p. 315-22.
12. Mauiyyedi, S. and R.B. Colvin, *Humoral rejection in kidney transplantation: new concepts in diagnosis and treatment*. Curr Opin Nephrol Hypertens, 2002. **11**(6): p. 609-18.
13. Takemoto, S.K., A. Zeevi, S. Feng, et al., *National conference to assess antibody-mediated rejection in solid organ transplantation*. Am J Transplant, 2004. **4**(7): p. 1033-41.
14. Gloor, J.M., S.R. DeGoey, A.A. Pineda, et al., *Overcoming a positive crossmatch in living-donor kidney transplantation*. Am J Transplant, 2003. **3**(8): p. 1017-23.
15. Faguer, S., N. Kamar, C. Guilbeaud-Frugier, et al., *Rituximab therapy for acute humoral rejection after kidney transplantation*. Transplantation, 2007. **83**(9): p. 1277-80.
16. Crespo, M., M. Pascual, N. Tolkoff-Rubin, et al., *Acute humoral rejection in renal allograft recipients: I. Incidence, serology and clinical characteristics*. Transplantation, 2001. **71**(5): p. 652-8.

17. Venetz, J.P. and M. Pascual, *New treatments for acute humoral rejection of kidney allografts*. Expert Opin Investig Drugs, 2007. **16**(5): p. 625-33.
18. Solez, K., R.B. Colvin, L.C. Racusen, et al., *Banff '05 Meeting Report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN')*. Am J Transplant, 2007. **7**(3): p. 518-26.
19. David-Neto, E., E. Prado, A. Beutel, et al., *C4d-positive chronic rejection: a frequent entity with a poor outcome*. Transplantation, 2007. **84**(11): p. 1391-8.
20. Worthington, J.E., A. McEwen, L.J. McWilliam, et al., *Association between C4d staining in renal transplant biopsies, production of donor-specific HLA antibodies, and graft outcome*. Transplantation, 2007. **83**(4): p. 398-403.
21. El-Zoghby, Z.M., M.D. Stegall, D.J. Lager, et al., *Identifying specific causes of kidney allograft loss*. Am J Transplant, 2009. **9**(3): p. 527-35.
22. Burton, S.A., N. Amir, A. Asbury, et al., *Treatment of antibody-mediated rejection in renal transplant patients: a clinical practice survey*. Clin Transplant, 2015. **29**(2): p. 118-23.
23. Moher, D., A. Liberati, J. Tetzlaff, et al., *Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement*. BMJ, 2009. **339**: p. b2535.
24. Downs, S.H. and N. Black, *The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions*. J Epidemiol Community Health, 1998. **52**(6): p. 377-84.
25. Deeks, J.J., J. Dinnes, R. D'Amico, et al., *Evaluating non-randomised intervention studies*. Health Technol Assess, 2003. **7**(27): p. iii-x, 1-173.

26. Howick, J., I. Chalmers, P. Glasziou, et al. *The Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence*. [cited 2016 8th August]; Available from: <http://www.cebm.net/ocebmllevels-of-evidence/>.
27. Hychko, G., A. Mirhosseini, A. Parhizgar, et al., *A systematic review and meta-analysis of rituximab in antibody-mediated renal allograft rejection*. Int J Org Transplant Med, 2011. **2**(2): p. 51-56.
28. Roberts, D.M., S.H. Jiang, and S.J. Chadban, *The treatment of acute antibody-mediated rejection in kidney transplant recipients-a systematic review*. Transplantation, 2012. **94**(8): p. 775-83.
29. Sautenet, B., G. Blanche, M. Buchler, et al., *One-year results of the effects of rituximab on acute antibody-mediated rejection in renal transplantation: RITUX ERAH, a multicenter double-blind randomized placebo-controlled trial*. Transplantation, 2016. **100**(2): p. 391-9.
30. Kaposztas, Z., H. Podder, S. Mauiyyedi, et al., *Impact of rituximab therapy for treatment of acute humoral rejection*. Clin Transplant, 2009. **23**(1): p. 63-73.
31. Lefaucheur, C., D. Nochy, J. Andrade, et al., *Comparison of combination plasmapheresis/IVIg/anti-CD20 versus high-dose IVIg in the treatment of antibody-mediated rejection*. Am J Transplant, 2009. **9**(5): p. 1099-107.
32. Liu, T.L., Y.G. Liu, M. Li, et al., *Clinical application of rituximab in antibody mediated rejection after renal transplantation*. Chinese Journal of Tissue Engineering Research, 2012. **16** (40): p. 7438-7443.
33. Steinmetz, O.M., F. Lange-Husken, J.E. Turner, et al., *Rituximab removes intrarenal B cell clusters in patients with renal vascular allograft rejection*. Transplantation, 2007. **84**(7): p. 842-850.

34. Redfield, R.R., T.M. Ellis, W. Zhong, et al., *Current outcomes of chronic active antibody mediated rejection - A large single center retrospective review using the updated BANFF 2013 criteria*. Hum Immunol, 2016. **77**(4): p. 346-52.
35. Chung, B.H., Y. Kim, H.S. Jeong, et al., *Clinical outcome in patients with chronic antibody-mediated rejection treated with and without rituximab and intravenous immunoglobulin combination therapy*. Transpl Immunol, 2014. **31**(3): p. 140-144.
36. Bachelet, T., C. Nodimar, J.-L. Taupin, et al., *Intravenous immunoglobulins and rituximab therapy for severe transplant glomerulopathy in chronic antibody-mediated rejection: a pilot study*. Clin Transplant, 2015. **29**(5): p. 439-46.
37. Smith, R.N., F. Malik, N. Goes, et al., *Partial therapeutic response to Rituximab for the treatment of chronic alloantibody mediated rejection of kidney allografts*. Transpl Immunol, 2012. **27**(2-3): p. 107-13.
38. Hychko, G., C. Hollenbeak, A. Parhizgar, et al., *A systematic review and meta-analysis of rituximab in refractory antibody-mediated renal allograft rejection*. Am J Transplant, 2010. **10**(Issue Supplement s4 - 2010 American Transplant Congress Abstracts): p. 237.
39. Sautenet, B., G. Blanche, M. Buchler, et al., *Ritux-erah: Multicenter randomized trial of rituximab on acute humoral rejection in transplantation*. Transpl Int, 2013. **26**(Issue s2 - Abstracts of the 16th Congress of the European Society for Organ Transplantation): p. 110.
40. Sautenet, B., G. Blanche, M. Buchler, et al., *Ritux-erah: Multicenter randomized trial of rituximab on acute antibody mediated rejection in transplantation*. Transpl Int, 2013. **26**(Issue s3 - Abstracts of the 13th Annual Congress of the French Speaking Society of Transplantation): p. 22.

41. Sautenet, B., G. Blanche, M. Buchler, et al., *One year results of the effects of rituximab on acute humoral rejection in renal transplantation: RITUX ERAH, a multicenter randomized placebo controlled trial*. Am J Transplant, 2013. **13**(Issue S5 - 2013 American Transplant Congress Abstracts): p. 112.
42. Gheith, O., T. Alotaibi, N. Nampoory, et al., *Early vs. late acute antibody mediated rejection among renal transplant recipients in terms of its response to rituximab therapy-single center experience*. Nephrol Dial Transplant, 2016. **31**(Supplement 1 - 53rd ERA-EDTA Congress Abstracts): p. i307.
43. Alotaibi, T., O. Gheith, N. Nampoory, et al., *Early vs. late acute antibody mediated rejection among renal transplant recipients in terms of its response to rituximab therapy-single center experience*. Transpl Int, 2015. **28**(Issue S4 - Abstracts of the 17th Congress of the European Society for Organ Transplantation): p. 547.
44. Gheith, O., T. Alotaibi, N. Nampoory, et al., *Early vs. late acute antibody mediated rejection among renal transplant recipients in terms of its response to rituximab therapy-single center experience*. Nephrol Dial Transplant, 2015. **30**(Supplement 3 - 52nd ERA-EDTA Congress Abstracts): p. iii644.
45. Nampoory, N., T. Alotaibi, O. Gheith, et al. *Early vs. Late acute antibody mediated rejection among renal transplant recipients in terms of its response to rituximab therapy-single center experience*. 15th American Transplant Congress Abstracts 2015 [cited 2016 8th August]; Available from: <http://www.atcmeetingabstracts.com/abstract/early-vs-late-acute-antibody-mediated-rejection-among-renal-transplant-recipients-in-terms-of-its-response-to-rituximab-therapy-single-center-experience/>.

46. Loupy, A., C. Lefaucheur, D. Vernerey, et al., *Outcome and therapeutic approaches in acute rejection with vascular lesions and DSAs*. Am J Transplant, 2011. **11**(Issue Supplement s2 - 2011 American Transplant Congress Abstracts): p. 193.
47. Gheith, O., T. Al-Otaibi, M.R.N. Nampoory, et al., *Acute antibody-mediated rejection in paediatric renal transplant recipients: Kuwait experience*. Exp Clin Transplant, 2014. **12**(Supplement N:2 - 14th Congress of the Middle East Society for Organ Transplantation Abstracts): p. 187.
48. Gheith, O., T. Al-Otaibi, N.M.R. Nampoory, et al., *Acute antibody-mediated rejection in paediatric renal transplant recipients: Single centre experience*. Transpl Int, 2013. **26**(Issue s2 - Abstracts of the 16th Congress of the European Society for Organ Transplantation): p. 330.
49. Redfield, R., T. Ellis, W. Zhong, et al. *Chronic active antibody mediated rejection (CABMR): Treatment, outcomes and predictors of graft loss in a large case series*. Abstracts from the 15th American Transplant Congress 2015 [cited 2016 8th August]; Available from: <http://www.atcmeetingabstracts.com/abstract/chronic-active-antibody-mediated-rejection-cabmr-treatment-outcomes-and-predictors-of-graft-loss-in-a-large-case-series/>.
50. Kim, Y., H.S. Kim, B.S. Choi, et al., *Comparative analysis of the effect of combination therapy with rituximab and intravenous immunoglobulin on the progression of chronic antibody mediated rejection in renal transplant recipients*. Nephrol Dial Transplant, 2014. **29**(Supplement 3 - 51st ERA-EDTA Congress Abstracts): p. iii324.

51. Anwar, S., R. Delos Santos, T. Horwedel, et al., *Rituximab does not prevent graft loss in renal transplant recipients with chronic antibody mediated rejection with significant renal impairment and nephrotic range proteinuria*. Transplantation, 2014. **98**(Supplement 1 - 2014 World Transplantation Congress Abstracts): p. 477.
52. Chaparro, A., M. Monteverde, Z. Balbarrey, et al., *Prevalence of HLA antibodies and C4D deposits in pediatric renal transplantation: Serologic/histologic correlations, impact on graft function and survival*. Pediatr Transplant, 2013. **17**(Issue Supplement s1 - International Pediatric Transplant Association 7th Congress on Pediatric Transplantation Abstracts): p. 45.
53. Bachelet, T., C. Nodimar, J.L. Taupin, et al., *Treatment of transplant glomerulopathy during chronic antibody-mediated rejection with intravenous immunoglobulin and rituximab*. Am J Transplant, 2012. **12**(Issue Supplement s3 - 2012 American Transplant Congress Abstracts): p. 413.
54. Ghouti-Terki, L., B. Sautenet, C. Barbet, et al., *Treatment of chronic antibody-mediated rejection after kidney transplantation: Does rituximab and intravenous globulins improve graft survival?* Am J Transplant, 2013. **13**(Issue S5 - 2013 American Transplant Congress Abstracts): p. 328.
55. Scemla, A., A. Loupy, S. Candon, et al., *Incidence of infectious complications in highly sensitized renal transplant recipients treated by rituximab: a case-controlled study*. Transplantation, 2010. **90**(11): p. 1180-1184.
56. Zarkhin, V., L. Li, N. Kambham, et al., *A randomized, prospective trial of rituximab for acute rejection in pediatric renal transplantation*. Am J Transplant, 2008. **8**(12): p. 2607-17.

57. Macaluso, J., M. Killackey, A. Paramesh, et al., *Comparative study of bortezomib therapy for antibody mediated rejection*. Am J Transplant, 2011. **11**(Issue Supplement s2 - 2011 American Transplant Congress Abstracts): p. 160.
58. Belliere, J., L. Rostaing, C. Guilbeau-Frugier, et al., *Low- versus high-dose rituximab for antibody-mediated rejection after kidney transplantation*. Transpl Int, 2013. **26**(2): p. e12-4.
59. Zarkhin, V., L. Li, N. Kambham, et al., *A randomized, prospective trial of rituximab for acute rejection in pediatric renal transplantation*. Pediatr Transplant, 2009. **13**(Issue Supplement s1 - 5th Congress of the International Pediatric Transplant Association Abstracts): p. 97.
60. Sarwal, M., V. Zarkhin, S. Mohile, et al., *Randomized trial of rituximab vs. standard of care for B cell dense acute renal transplant rejection*. Am J Transplant, 2007. **7**(Issue Supplement s2 - 7th American Transplant Congress Abstracts): p. 287.
61. Kamar, N., O. Milioto, B. Puissant-Lubrano, et al., *Incidence and predictive factors for infectious disease after rituximab therapy in kidney-transplant patients*. Am J Transplant, 2010. **10**(1): p. 89-98.
62. Chung, B.H., J.T. Yun, S.E. Ha, et al., *Combined use of rituximab and plasmapheresis pre-transplant increases post-transplant infections in renal transplant recipients with basiliximab induction therapy*. Transpl Infect Dis, 2013. **15**(6): p. 559-68.
63. van Vollenhoven, R.F., R.M. Fleischmann, D.E. Furst, et al., *Longterm Safety of Rituximab: Final Report of the Rheumatoid Arthritis Global Clinical Trial Program over 11 Years*. J Rheumatol, 2015. **42**(10): p. 1761-6.

Figures and Tables

Figure 1 – Flow diagram to show inclusion and exclusion of articles for this review

Table 1 – Outcomes of primary studies of rituximab as treatment for acute antibody-mediated rejection (AAMR)

Table 2 – Outcomes of primary studies of rituximab as treatment for chronic antibody-mediated rejection (CAMR)

Table 1 – Outcomes of primary studies of rituximab as treatment for acute antibody-mediated rejection (AAMR)								
Study (Year) Country	Inclusion Criteria	Number of Ptx (RTX/non-RTX)	Study Period (months)	Tx Regimen (RTX/non-RTX)	% Ptx Survival (RTX/non-RTX)	% Graft Survival (RTX/non-RTX)	Graft Function	Adverse Effects
Randomized controlled trials								
Sautenet (2016) France [29, 39-41]	Bx-proven AAMR	38 (19/19)	12	RTX, PE + IVIg/ Placebo, PE, + IVIg All ptx received CS, MMF + TAC	No significant difference 100.0/100.0 ^a	No significant difference 94.7/94.7 ^a	No significant difference	No statistical comparison
Retrospective cohort studies								
Nampoory (2015)* Kuwait [42-45]	AAMR ^b	103 (65/38)	Not reported ^c	RTX, PP + IVIg/ PP + IVIg Additional Tx not reported	No significant difference Actual numbers not reported (P>0.05)	Favours RTX Actual numbers not reported (P=0.028)	Not reported	No significant difference
Kaposztas (2009) USA [30]	Bx-proven AAMR	54 (26/28)	24	RTX, PP ± IVIg/ PP ± IVIg All ptx received CS ± CsA ± MMF ± SRL	No significant difference 96.2/89.3 (P=NS)	Favours RTX 88.5/60.7 (P=0.005)	No significant difference	No significant difference ^d
Lefaucher (2009) France [31, 46]	Bx-proven AMR	24 (12/12)	36	RTX, PP + IVIg/ IVIg All ptx received CS ± Switch	No statistical comparison 100.0/91.7	Favours RTX 91.7/50.0 (P=0.02)	No significant difference	No significant difference
Gheith (2013)*	Pediatric bx-proven	20 (Not reported)	Not reported	RTX, PE + IVIg/ Not reported ^c	Not reported	Favours RTX	Not reported	Not reported

Kuwait [47, 48]	AAMR or mixed AR			All ptx received CS, MMF + TAC		Actual numbers not reported (P=0.002)		
Liu (2012) China [32]	Bx-proven AAMR	18 (8/10)	12	RTX, ATG ± PE/ ATG All ptx received CS, MMF + TAC	No significant difference 100.0/100.0	No statistical comparison 100.0/80.0	Favours RTX	No statistical comparison
Steinmetz (2007) Germany [33]	Bx-proven AVR	11 (5/6) ^f	3	RTX/ Nil Additional Tx was variable ^g	No significant difference 100.0/100.0	No significant difference 100.0/100.0	No statistical comparison	No significant difference

All outcomes are reported for the end of the study period unless stated otherwise (P value reported as in publication, when available)

* Abstract only

^a Results of intention-to-treat analysis

^b Diagnostic criteria not reported

^c Overall study period is ten years but the length of follow-up is not reported

^d At six-month follow-up

^e Comparison Tx regimens not reported but some patients received lymphocyte depleting agents and experienced significantly worse graft survival (P=0.036)

^f Although the study includes 16 ptx, only the C4d positive cases have been analysed in this review

^g In the RTX group, most ptx also received CS + Switch with one ptx receiving PP + IVIg; in the non-RTX group, most ptx received CS + Switch ± ATG with one ptx receiving IVIg and another switching from a calcineurin inhibitor to the mTOR inhibitor everolimus

(A)AMR – (acute) antibody-mediated rejection; AR – acute rejection; ATG – anti-thymocyte globulin; AVR – acute vascular rejection; Bx – biopsy; CS – corticosteroids; CsA – ciclosporin A; IVIg – intravenous immunoglobulin; MMF – mycophenolate mofetil; mTOR – mechanistic target of rapamycin; NS – non-significant; PE – plasma exchange; PP – plasmapheresis; Ptx – patient; RTX – rituximab; SRL – sirolimus; Switch – switch from ciclosporin A to tacrolimus; TAC – tacrolimus; Tx – treatment; USA – United States of America

Table 2 – Outcomes of primary studies of rituximab as treatment for chronic antibody-mediated rejection (CAMR)								
Study (Year) Country	Inclusion Criteria	Number of Ptx (RTX/non-RTX)	Study Period (months)	Tx Regimen (RTX/non-RTX)	% Ptx Survival (RTX/non-RTX)	% Graft Survival (RTX/non-RTX)	Graft Function	Adverse Effects
<i>Retrospective cohort studies</i>								
Redfield (2016) USA [34, 49]	Bx-proven CAMR	123 (37/86)	24	RTX + variable/ Variable ^a All ptx received CS, MMF + CsA or TAC	Not reported	No significant difference Actual numbers not reported (P=NS)	Not reported	Not reported
Chung (2014) Republic of Korea [35, 50]	Bx-proven CAMR	54 (25/29)	36	RTX + IVIg/ Nil All ptx received CS + CsA or TAC	Not reported	Favours RTX 88.0/41.4 (P=0.03)	Favours RTX	No statistical comparison
Anwar (2014)* USA [51]	Bx-proven CAMR	39 (9/30)	<100 ^b	RTX/ Not reported All ptx received CS, MMF + TAC	Not reported	Favours non-RTX 22.2/70.0 (P<0.001)	Not reported	Not reported
Chaparro (2013)* Argentina [52]	Bx-proven pediatric CAMR	32 (8/24)	~ 59 ^c	RTX + IVIg/ IVIg All ptx received CS, MMF + CsA, SRL or TAC	Not reported	Not reported	No significant difference	Not reported
Bachelet (2015) France [36, 53]	Bx-proven CAMR-associated TG	31 (21/10)	24	RTX, IVIg + CS/ Nil All ptx received MMF or AZA + CsA or TAC	No significant difference 95.2/100.0 (P=0.48)	No significant difference 47.6/40.0 (P=0.69)	No significant difference	Favours non-RTX

Smith (2012) USA [37]	Bx-proven CAMR	31 (14/17)	~ 66 ^d	RTX ± PP ± IVIg ± ACT ± ATG/ Variable ^e RTX group also received CS, MMF + TAC	No statistical comparison 92.9/82.4	No significant difference 28.6/0.0 (P=0.05)	No statistical comparison	No statistical comparison
Ghouti-Terki (2013)* France [54]	Bx-proven CAMR	22 (8/14)	12	RTX, PE + IVIg/ CS Additional Tx not reported	Not reported	No significant difference Actual numbers not reported (P=0.27)	Favours non-RTX	Not reported
<p>All outcomes are reported for the end of the study period unless stated otherwise (P value reported as in publication, when available)</p> <p>* Abstract only</p> <p>^a Study ptx were treated with a variable combination of CS (93%), IVIg (87%), RTX (30%), PE (13%) and ATG (10%); 7% ptx received no Tx</p> <p>^b Follow-up of 150 months for non-RTX group, less than 100 months' follow-up available for RTX group</p> <p>^c Median follow-up of 4.9 years</p> <p>^d Follow-up of 2000 days</p> <p>^e Control group received minor modifications to their immunosuppression including switch from AZA to MMF (41%) and a mixture of CS, CsA or TAC and, in one ptx, IVIg</p> <p><i>ACT – actinomycin; ATG – anti-thymocyte globulin; AZA – azathioprine; Bx – biopsy; CAMR – chronic antibody-mediated rejection; CS – corticosteroids; CsA – ciclosporin A; IVIg – intravenous immunoglobulin; MMF – mycophenolate mofetil; NS – non-significant; PE – plasma exchange; PP – plasmapheresis; Ptx – patient; RTX – rituximab; SRL – sirolimus; TAC – tacrolimus; TG – transplant glomerulopathy; Tx – treatment; USA – United States of America</i></p>								

Figure legend

Figure 1 – Flow diagram to show inclusion and exclusion of articles for this review.

AMR = antibody-mediated rejection

Supplementary Material 1 – Search Strategies

The Cochrane Library

The population – renal transplant recipients	
1	TRANSPLANTATION (MeSH term, this term only)
2	ORGAN TRANSPLANTATION (MeSH term, this term only)
3	exp KIDNEY TRANSPLANTATION (MeSH term, this term only)
4	((kidney* or renal* or organ* or viscera*) NEAR/5 transplant*):ti,ab
5	((kidney* or renal* or organ* or viscera*) NEAR/5 graft*):ti,ab
6	((kidney* or renal* or organ* or viscera*) NEAR/5 allograft*):ti,ab
7	#1 OR #2 OR #3 OR #4 OR #5 OR #6
The intervention – rituximab	
8	(rituximab or mabthera or rituxan or CD20 or C2B8):ti,ab
9	#7 AND #8

OVID Embase

The population – renal transplant recipients	
1	TRANSPLANTATION/
2	ORGAN TRANSPLANTATION/
3	exp KIDNEY TRANSPLANTATION/
4	((kidney\$1 or renal\$1 or organ\$1 or viscera\$) adj5 transplant\$).ti,ab.
5	((kidney\$1 or renal\$1 or organ\$1 or viscera\$) adj5 graft\$).ti,ab.
6	((kidney\$1 or renal\$1 or organ\$1 or viscera\$) adj5 allograft\$).ti,ab.
7	or/1-6
The intervention – rituximab	
8	RITUXIMAB/
9	(rituximab or mabthera or rituxan or CD20 or C2B8).ti,ab.
10	or/8-9
11	7 AND 10

OVID MEDLINE

The population – renal transplant recipients	
1	TRANSPLANTATION/
2	ORGAN TRANSPLANTATION/
3	exp KIDNEY TRANSPLANTATION/
4	((kidney\$1 or renal\$1 or organ\$1 or viscera\$) adj5 transplant\$).ti,ab.
5	((kidney\$1 or renal\$1 or organ\$1 or viscera\$) adj5 graft\$).ti,ab.
6	((kidney\$1 or renal\$1 or organ\$1 or viscera\$) adj5 allograft\$).ti,ab.
7	or/1-6
The intervention – rituximab	
8	(rituximab or mabthera or rituxan or CD20 or C2B8).ti,ab.
9	7 and 8

The Transplant Library

The intervention – rituximab	
1	(rituximab or mabthera or rituxan or CD20 or C2B8).ti,ab.

Supplementary Material 2 – Study Quality Assessment

Study (Year)	Question																											Rep (11)	EV (3)	IV-B (7)	IV-C (6)	Pow (5)	Total (32)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27						
Bachelet (2015)	1	1	1	1	2	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	0	0	0	1	1	0	11	3	4	3	0	21	
Chung (2014)	1	1	1	1	2	1	1	1	1	0	1	1	1	0	0	0	1	1	1	1	0	0	0	1	1	0	10	3	4	3	0	20	
Kaposztas (2009)	1	1	1	1	2	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	0	0	0	1	1	0	11	3	4	3	0	21	
Lefaucher (2009)	1	1	1	1	2	1	1	0	1	0	1	1	1	0	0	0	1	1	1	1	1	0	0	0	1	1	0	9	3	4	3	0	19
Liu (2012)	1	1	1	1	2	1	1	1	1	0	1	1	1	0	0	0	1	1	1	1	1	0	0	0	1	1	0	10	3	4	3	0	20
Redfield (2016)	1	1	1	1	0	1	1	0	1	1	1	1	1	0	0	0	1	1	1	1	1	0	0	1	1	0	8	3	4	4	0	19	
Sautenet (2016)	1	1	1	1	2	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	11	2	7	6	0	26	
Smith (2012)	1	1	1	1	2	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	0	0	1	1	0	11	3	4	3	0	21	
Steinmetz (2007)	1	0	1	1	2	1	1	0	1	1	1	1	1	0	0	0	1	1	1	1	1	0	0	1	1	0	9	3	4	4	0	20	
EV – external validity; IV-B – internal validity (bias); IV-C – internal validity (confounding); Pow – power; Rep – reporting																																	