Rituximab-Related Late-Onset Neutropenia in Kidney Transplant Recipients Treated for Antibody-Mediated Acute Rejection

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Abstract

Objectives: Kidney transplant is a new area for use of rituximab, which is being used to treat acute antibody-mediated rejection or as an induction agent in ABO- or HLA-incompatible grafts. We report on late-onset neutropenia in rituximab-treated kidney transplant recipients with antibody-mediated rejection.

Materials and Methods: This observational prospective study was performed on kidney transplant recipients with clinically suspicious or biopsy-proven antibody-mediated rejection treated with plasmapheresis plus intravenous immunoglobulin with (cases) or without (controls) rituximab.

Results: Compared with none of the controls, 4 of 6 patients (66.7%) in the rituximab-treated group experienced late-onset neutropenia 35 to 93 days after the last dose of rituximab. The course of neutropenia was complicated by endocarditis in 1 patient, resulting in his death just because of a lack of valvular surgery.

Conclusions: Increased use of rituximab to treat antibody-mediated rejection among kidney transplant recipients requires attention to its late-onset adverse event, neutropenia. Although asymptomatic in some patients, kidney transplant recipients treated concomitantly with plasmapheresis and mycophenolate mofetil are predisposed to hypogammaglobulinemia, and monitoring of patients for infections is required.

Key words: Acute antibody-mediated rejection, Kidney transplant, Late-onset neutropenia

Introduction

Rituximab, a chimeric monoclonal antibody against CD20 antigens on B lymphocytes, was first introduced to treat CD20-positive non-Hodgkin lymphoma.1 It is used now for some autoimmune diseases such as rheumatologic diseases,2-4 Pemphigus,5 and acute antibody-mediated rejection (AMR) in kidney transplant recipients.6 A recently recognized delayed adverse event of rituximab is late-onset neutropenia (LON). Rituximab-related LON has been defined as an absolute neutrophil count (ANC) of less than 1500/μL (grade 2 to 4 neutropenia based on National Cancer Institute Common Toxicity Criteria) appearing about 4 weeks after the last dose of rituximab administration in the absence of any alternative cause of neutropenia. Late-onset neutropenia incidence in lymphoma patients has been reported to be 3% to 27%, whereas in patients with autologous stem cell transplant higher rates of 42% to 70% have been noted.7,8 There are some case reports about rituximab-induced LON in patients with autoimmune diseases such as patients with rheumatoid arthritis3,4,9,10 and Pemphigus.11 Tesfa and associates have reported varying incidences of rituximab-related LON between patients with different autoimmune diseases. They have reported that, among patients with rheumatologic diseases, the incidence of rituximab LON is higher among patients with Wegener granulomatosis (23%) and systemic lupus erythematosus (20%) compared with patients with rheumatoid arthritis (3%)12; conversely, 2 other studies have shown low LON incidences of 3%3 and 5%4 among rituximab-treated patients with antineutrophil cytoplasmic antibody-associated vasculitis.4

The reported median time to onset of LON is 38 to 175 days after rituximab therapy termination, with
LON duration of 5 to 77 days. In most studies that reported LON, median number of doses of rituximab was 6, with range of 3 to 8 doses.

Although controversial, some possible risk factors have been proposed for rituximab-related LON including history of autologous stem cell transplant or human immunodeficiency virus-related lymphoma, previous chemotherapy, receiving high cumulative doses of rituximab, previous administration of purine analogs, or high-dose methotrexate.

It has been found that LON patients have persistent and intense B-lymphocyte depletion and low serum IgM concentrations after rituximab administration. Late-onset neutropenia is possibly the consequence of competition between B-cell lymphopoiesis and neutropoiesis. During B-cell recovery in the bone marrow, there is promotion of B-cell lymphopoiesis over granulopoiesis. Low granulopoiesis concurs with high levels of B-cell activating factor, an antiapoptotic factor that stimulates B-cell recovery.

Kidney transplant is a new area for use of rituximab, which is used to treat AMR or as induction agent in ABO- or HLA-incompatible kidney transplants. There is a paucity of reports on rituximab-related LON in kidney transplant recipients who have received rituximab for different indications. Here, we report our experiences with LON in rituximab-treated kidney transplant recipients with AMR.

Materials and Methods

Study design and setting
This observational, prospective, case-control study was done at the kidney transplant ward of the Imam-Khomeini Hospital Complex affiliated with Tehran University of Medical Sciences from August 2014 to December 2015.

Patients and immunosuppressive protocols
Patients included kidney transplant recipients with highly clinically suspicious or biopsy-proven AMR who had been treated with plasmapheresis plus intravenous immunoglobulin (IVIg) with or without rituximab.

According to our protocol, all patients received thymoglobulin induction with cumulative doses of 4 to 5 mg/kg and a maintenance immunosuppressive regimen of prednisolone, tacrolimus (with desired whole blood trough concentration of 8-10 ng/mL), and mycophenolate mofetil (1-1.5 g/d based on leukocyte count). All patients received ganciclovir/valganciclovir, cotrimoxazole, and oral clotrimazole as prophylaxis for cytomegalovirus, Pneumocystis jiroveci pneumonia, and candidiasis. Ganciclovir/valganciclovir and cotrimoxazole doses were adjusted according to patient kidney function.

Depending on the choice of nephrologist, AMR episodes were treated with plasmapheresis plus IVIg (considered controls) or plasmapheresis + IVIg + rituximab (considered cases). The patients usually underwent plasmapheresis every other day followed by a dose of 100 mg/kg IVIg after each plasmapheresis session. When the nephrologist decided to add rituximab to the AMR regimen, the rituximab was given as 4 weekly doses of 375 mg/m² on plasmapheresis days, after plasmapheresis, and 1 to 2 hours after completion of IVIg infusion.

Late-onset neutropenia definition
Based on the National Cancer Institute Common Toxicity Criteria, neutropenia is defined as ANC of less than 1500/μL and classified as grade 2, grade 3, and grade 4 for ANC of less than 1500, less than 1000, and less than 500/μL. Late-onset neutropenia was considered when neutropenia occurred about 4 weeks after the last dose of rituximab had been given without any other alternate explanation (eg, unresponsive to dose reduction/cessation of ganciclovir/valganciclovir, or mycophenolate mofetil).

Ethics
The study protocol was approved by the local Ethics Committee of Tehran University of Medical Sciences. All of the protocols conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from all participants.

Statistical analyses
Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 17.0, IBM Corporation, Armonk, NY, USA). Results are given as the median (range) and proportion for continuous and categorical variables.

Results
Based on AMR treatment protocols ordered by the nephrologists, 6 patients (4 men and 2 women)
received plasmapheresis + IVIg + rituximab and were evaluated as “cases,” while 5 patients (2 women and 3 men) were treated with plasmapheresis + IVIg and considered “controls.” The median observation period of patients was 9 months (range, 6-15 mo).

Demographic, clinical, and some related laboratory data of patients are shown in Table 1. In all rituximab-treated patients, the first rituximab dose was given at median time of 5 days (range, 4-32 d) after thymoglobulin termination, and none of the patients had leukopenia before rituximab initiation that had been attributable to thymoglobulin administration (Figure 1). Compared with none of the control patients, 4 of 6 patients (66.7%) in the rituximab-treated group experienced LON 35 to 93 days after the last dose of rituximab. All patients who experienced LON were men, while none of the women showed LON. Two patients showed grade 2 and two grade 3 neutropenia (Table 2).

Platelet counts in patients with LON were in the range of 180 to 300 × 10³/μL during neutropenia. The range of platelet counts in the rituximab-treated patients who had not experienced LON and in controls were both 150 to 200 × 10³/μL. These data on platelets ruled out bone marrow suppression.

**Table 1. Demographic, Clinical, and Laboratory Data of Cases and Controls**

<table>
<thead>
<tr>
<th>Case Group, No. of Patients or Median (Range)</th>
<th>Late-Onset Neutropenia (+)</th>
<th>Late-Onset Neutropenia (-)</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>6</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Sex (women/men)</td>
<td>2/4</td>
<td>0/4</td>
<td>2/3</td>
</tr>
<tr>
<td>Age, y</td>
<td>30.5 (19-50)</td>
<td>30.5 (19-40)</td>
<td>34.5 (19-50)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>45.5 (42-58)</td>
<td>47.5 (42-58)</td>
<td>44 (42-46)</td>
</tr>
<tr>
<td>No. of retransplants</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Donor age, y</td>
<td>26 (18-45)</td>
<td>26 (25-40)</td>
<td>31.5 (18-45)</td>
</tr>
<tr>
<td>Donor sex (women/men)</td>
<td>3/3</td>
<td>1/3</td>
<td>33 (14-58)</td>
</tr>
<tr>
<td>Donor serum Cr, mg/dL</td>
<td>0.95 (0.7-1.5)</td>
<td>0.8 (0.7-0.9)</td>
<td>1.25 (1-1.5)</td>
</tr>
<tr>
<td>HD duration, mo</td>
<td>24 (18-28)</td>
<td>276 (24-282)</td>
<td>21 (18-24)</td>
</tr>
<tr>
<td>ESRD cause</td>
<td>HTN: 1</td>
<td>Recurrent stone: 2</td>
<td>Recurrent ston: 1</td>
</tr>
<tr>
<td></td>
<td>Reflux nephropathy: 1</td>
<td>Unknown: 1</td>
<td>Unknown: 1</td>
</tr>
<tr>
<td></td>
<td>MMF daily dose</td>
<td>0.5-1.5g</td>
<td>1.5 g: 2</td>
</tr>
<tr>
<td></td>
<td>Ganciclovir dose</td>
<td>Regularly adjusted for patients based on their creatinine clearance</td>
<td>1-1.5 g</td>
</tr>
<tr>
<td>No. of plasmapheresis sessions</td>
<td>11 (7-17)</td>
<td>12 (7-17)</td>
<td>7 (5-15)</td>
</tr>
</tbody>
</table>

**Table 2. Late-Onset Neutropenia Experienced by Rituximab-Treated Patients**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Time To Onset of LON, d</th>
<th>LON Duration, d</th>
<th>Nadir WBC (Nadir ANC) Before G-CSF Therapy (cells/μL)</th>
<th>Treatment With G-CSF, d</th>
<th>Infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>93</td>
<td>Unknown (patient died)</td>
<td>1100 (880)</td>
<td>Yes (9)</td>
<td>Endocarditis</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>28</td>
<td>1200 (840)</td>
<td>Yes (5)</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>110</td>
<td>1300 (1000)</td>
<td>Yes (6)</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>35</td>
<td>1200 (1000)</td>
<td>Yes (1)</td>
<td>No</td>
</tr>
</tbody>
</table>

**Abbreviations:** ADPKD, autosomal dominant polycystic kidney diseases; Cr, creatinine; ESRD, end-stage renal diseases; HD, hemodialysis; HTN, hypertension; LON, late-onset neutropenia; MMF, mycophenolate mofetil.

Control group received plasmapheresis + IVIg treatment; case group received plasmapheresis + IVIg + rituximab.

**Figure 1. Absolute Neutrophil Counts Pretransplant, After Transplant (Thymoglobulin Effect), Before Rituximab Administration, and After Rituximab Administration**

Abbreviations: ANC, absolute neutrophil count; ATG, antithymocyte globulin; G-CSF, granulocyte colony-stimulating factor; LON, late-onset neutropenia. The last point on the graph for each patient is the ANC the patient had at the end of neutropenia. Increases in ANC after LON occurrence were because of G-CSF administration.

*Median time between ATG discontinuation and administration of the first dose of rituximab was 5 days (range, 4-32 d).*
because of ganciclovir/valganciclovir or mycophenolate mofetil.

The median time of hemodialysis treatment before transplant was higher among patients with LON (276 mo) than in rituximab-treated patients who did not show LON (21 mo) or controls (22 mo). All patients who experienced LON were treated with granulocyte-colony stimulating factor (G-CSF); except for 1 patient who died, all other 3 patients recovered from LON after G-CSF administration. In all patients, within 1 day after G-CSF administration, leukocyte counts increased and neutropenia resolved. The highest leukocyte count was achieved soon after G-CSF therapy in 1 patient whose leukocyte and ANC counts increased to 12 600 and 8820/μL after 3 daily doses of G-CSF. In the other 3 patients, leukocyte counts were raised to 3000/μL (ANC = 2100/μL). The leukocyte count was maintained at 2000 to 2500/μL (ANC = 1600-2000/μL) in 2 patients and 4000 to 5000/μL (ANC = 3200-4000/μL) in the other 2 patients after completing LON. During the neutropenic period, G-CSF effects lasted for a maximum of 1 week, and leukocyte counts decreased thereafter. When ANC began to drop under 1000/μL, G-CSF was administered until the end of neutropenia. The neutropenia course was complicated by endocarditis in 1 patient, resulting in his death just because of his nonpermission for valvular surgical procedure.

Discussion

Late-onset neutropenia has been reported as a delayed-onset adverse event of rituximab during the past several years (since 2007), mostly from patients with lymphoma9,8 and rheumatologic diseases.9,10,12 There are few reports on rituximab-related LON in kidney transplant recipients14-18 with some of these patients being given rituximab for desensitization before ABO- or HLA-incompatible transplant.14,15 This is the first study to compare LON in kidney transplant recipients who had been treated with rituximab for acute antibody-mediated rejection versus control patients, which is important due to the emerging area of rituximab administration in kidney transplant patients. Rituximab, in combination with plasmapheresis and IVIg, is a recommended treatment regimen for treating AMR in case series.17,19-21

In our observation, 4 of 6 rituximab-treated AMR patients (66.7%) experienced grade 2 or 3 neutropenia, while no patients who had been treated with plasmapheresis + IVIg became neutropenic despite similar maintenance immunosuppressive therapy and bacterial/viral/fungal prophylaxis. The incidence of LON with rituximab is estimated to be between 3% and 27% in previous studies7,8 among lymphoma and rheumatologic diseases patients and 42% to 70% among autologous stem-cell transplant patients receiving rituximab before or after transplant.7,8 This adverse event seems to be underestimated because most studies are retrospective and patients were not followed regularly for blood cell counts for considerable time after rituximab treatment. This lack of follow-up may result in unrecognized patients who were asymptomatic during their neutropenic course.7,8

There are 2 reports on rituximab-related LON among kidney transplant recipients who were treated with rituximab for ABO- or HLA-incompatible kidney transplant.14,15 In the study by Ishida and associates, 22 of 52 (42%) low-dose rituximab-treated patients showed LON compared with 15 of 68 (22%) rituximab-untreated patients.14 In that study, the mean time to rituximab-induced LON and LON duration was 4 to 6 months and 2 to 3 months, which is comparable with that reported in lymphoma patients.14 In the study by Kabei and associates, LON occurred in 12 of 25 (48%) kidney transplant recipients who received 1 to 2 low doses of 150 mg/m² of rituximab for desensitization.15 One case report16 and 2 case series17,18 on kidney transplant recipients who received rituximab for humoral rejection reported rituximab-induced neutropenia in 37.5% (3 of 8 patients) and 33.3% (3 of 9 patients) without using the word LON. These reports on AMR-treated kidney transplant patients had a lack of a control group. One possible reason for the higher rate of LON in these kidney transplant recipients may have been the concomitant use of other drugs that induce neutropenia such as mycophenolate mofetil, tacrolimus,15 cotrimoxazole, or performing plasmapheresis in these patients.14 In our study, the prevalence of LON among AMR-treated patients was 66.7%, which was higher than those previously reported in kidney transplant recipients.14,15 The main reason for this might have been that higher rituximab dosages were used to treat AMR in our patients compared with previous studies.14,15

In our observation, the median time to the onset of neutropenia was 58 days (35-93 d) from the
last dose of rituximab administration. This time is reported to have been between 38 and 175 days according to existing literature from lymphoma and rheumatic disease patients\(^7,8\) and 4 to 6 months in kidney transplant recipients who had been given rituximab for induction/desensitization.\(^{14}\) As seen, time to LON was shorter in our patients. Although the number of patients is too limited to draw a precise conclusion, AMR patients may be prone to more prevalent and earlier rituximab-related LON.

Although 1 patient died because of endocarditis, the other neutropenic patients reported in our study were asymptomatic and had been recognized in routine blood cell monitoring. The patient who died had valvular disturbances in his heart before transplant. Because of fear of the possible effects of surgery on his allograft and the risk of graft loss, the patient did not accept surgery and 2 months after the endocarditis diagnosis, he died despite antibiotic therapy.

Although rituximab-related LON has been reported as asymptomatic and self-limited in most patients, among 106 patients with LON who had been followed up until 2010, eighteen infection episodes (16.9\%) had been reported, ranging from 0\% to 20\% in different reviewed studies.\(^8\) More serious infections have been reported in patients with rheumatologic diseases who were concomitantly treated with immunosuppressive agents during neutropenia.\(^7\) As emphasized, infectious risk in neutropenic patients is associated with severity and duration of neutropenia.\(^7,8\) In patients with hypo IgG-emia, rituximab-induced LON may predispose patients to more infections.\(^8\) Hypo IgG-emia has been predicted in kidney transplant recipients who have been treated with mycophenolate mofetil. Unfortunately, in this study, we did not have the patients' immunoglobulin levels.

Although specific sex has not been mentioned as a risk factor for rituximab-related LON,\(^7,8\) all 4 patients who experienced LON in our study were men. None of the 2 women treated with rituximab showed LON. Based on our observation, with the limited number of rituximab-administered kidney transplant recipients, longer hemodialysis treatment before transplant may predispose patients to rituximab-related LON after a kidney transplant. This hypothesis must be assessed in larger studies.

Conclusions

Increased use of rituximab to treat AMR among kidney transplant recipients necessitates attention to its delayed onset adverse event, neutropenia. Although asymptomatic in some patients, kidney transplant recipients with AMR who are usually treated concomitantly with plasmapheresis and mycophenolate mofetil may be predisposed to hypogammaglobulinemia. Monitoring for infectious episodes is necessary.

References


