ABSTRACT

Objective: To evaluate the incidence of antibody-mediated rejection after the C4d and donor specific antibody detection was provided by Luminex in renal transplantation biopsies; to compare acute antibody-mediated rejection characteristics as related to acute cellular rejection; to evaluate the impact on the incidence of acute antibody mediated rejection after the utilization of cross match test by flux cytometry and the detection of pre-transplantation donor specific antibody in patients with previous history of exposition to alloantigens. Methods: One hundred twenty-four renal transplanted patients were evaluated through the detection of C4d in early biopsies of those presenting graft dysfunction and the detection of antibody against donor when C4d was positive. The acute antibody mediated rejection was treated by plasmapheresis and intravenous immunoglobulin. Results: The incidence of acute rejection was 18.8%, being the acute cellular rejection 14.9% and acute antibody mediated rejection 6.6%. When both were compared, the acute antibody-mediated rejection were earlier than the acute cellular rejection (12.5 versus 59.9 days, p = NS), being more frequent in female patients (75 versus 29%, p = 0.05), with deceased donors (75 versus 33%, p = 0.09), with higher dialysis time (87.7 versus 47.4, p = 0.03), greater number of transfusion episodes (4.6 versus 1.4, p = 0.02), greater panel reaction activity (28.0 versus 4.8, p = 0.03) and more frequently in re-transplanted patients (50 versus 5.6%, p = 0.02). Delayed graft function was more frequent in antibody mediated rejection (100 versus 50%, p = 0.02). All patients with acute cellular rejection reversed graft function after treatment, with 100% graft survival after one year. Among patients with acute antibody-mediated rejection, the treatment with plasmapheresis and immunoglobulin was efficient in reducing the titers of donor specific antibody (2605 versus 202 mfi, p < 0.001), but 3/8 of patients evolved to graft loss, making graft survival of 62.5% (p < 0.001). Conclusions: The routine use of detecting C4d and donor specific antibody increased the incidence of acute rejection. Acute antibody-mediated rejection presented clinical profile and therapeutic response different from acute cellular rejection, identifying a worse prognosis as well as therapeutic success.

Keywords: Graft rejection/diagnosis; Graft rejection/therapy; HLA antigens; Kidney transplantation; Plasmapheresis

RESUMO

Objetivo: Avaliar a incidência da rejeição mediada por anticorpo depois de instituída a pesquisa de C4d em biópsias de rim transplantado e pesquisa de anticorpo específico de doador pelo Luminex; comparar as características da rejeição aguda mediada por anticorpo em relação à rejeição aguda celular; avaliar o impacto na incidência de rejeição aguda mediada por anticorpo após utilizar a prova cruzada por citometria de fluxo e pesquisa de anticorpo específico de doador pré-transplante em pacientes

Study carried out at Hospital Israelita Albert Einstein – HIAE, São Paulo (SP), Brazil.
1 PhD student at Universidade Federal de São Paulo – UNIFESP, São Paulo (SP), Brazil; Assistant physician of Renal Transplantation Program at Hospital Israelita Albert Einstein – HIAE, São Paulo (SP), Brazil.
2 PhD; MD of Hospital Israelita Albert Einstein – HIAE, São Paulo (SP), Brazil.
3 MD of Hospital Israelita Albert Einstein – HIAE, São Paulo (SP), Brazil.
4 Assistant physician of Renal Transplantation Program of Hospital Israelita Albert Einstein – HIAE, São Paulo (SP), Brazil.
5 In memoriam; Post-doctorate degree; Full professor of the Department of Urology of Faculdade de Medicina do ABC – FMABC, Santo André (SP), Brazil.
6 MD of Hospital Israelita Albert Einstein – HIAE, São Paulo (SP), Brazil.
7 PhD; MD of Hospital Israelita Albert Einstein – HIAE, São Paulo (SP), Brazil.
8 PhD; MD of Hospital Israelita Albert Einstein – HIAE, São Paulo (SP), Brazil.
9 Post-doctorate degree; Adjunct professor of Universidade Federal de São Paulo – UNIFESP, São Paulo (SP), Brazil.
Corresponding author: Lúcio Roberto Requião Moura – Rua Botucatu, 740 – Vila Clementino – CEP 04023-900 – São Paulo (SP), Brasil – Tel.: (11) 3477-7229 – e-mail: lrequiao@nefro.epm.br
Received on: May 14, 2009 – Accepted on: Oct 16, 2009
com histórico de exposição prévia à aloantígenos. **Métodos:** Foram avaliados 124 pacientes transplantados renais, com pesquisa de C4d em biópsias precoces em pacientes com disfunção do enxerto e pesquisa de anticorpo contra o doador quando o C4d foi positivo. A rejeição aguda mediada por anticorpo foi tratada com plasmapherese e imunoglobulina intravenosa. **Resultados:** Foi encontrada uma incidência de rejeição aguda de 18,8%, com frequência de episódios de rejeição aguda celular de 14,9% e de rejeição aguda mediada por anticorpo de 6,6%. Quando comparados com rejeição aguda celular, os episódios de rejeição aguda mediada por anticorpo foram mais precoces (12,5 versus 59,9 dias, p = NS), sendo mais frequentes em pacientes femininas (75% versus 29%, p = 0,05), com doadores falecidos (75% versus 33%, p = 0,09), com maior tempo de diálise (87,7 versus 47,4, p = 0,03), com maior número de transfusões (4,6 versus 1,4, p = 0,02), com maior atividade contra painel (28,0 versus 4,8, p = 0,03) e mais frequentemente retransplantados (50 versus 5,6%, p = 0,02). Função retardada do enxerto foi mais frequente nos pacientes com rejeição aguda mediada por anticorpo (100 versus 50%, p = 0,02). Todos os pacientes com rejeição aguda celular reverteram a função do enxerto após o tratamento, com sobrevida do enxerto, em um ano, de 100%. Entre os pacientes com rejeição aguda mediada por anticorpo, o tratamento com plasmapherese e imunoglobulina foi eficiente em reduzir os títulos de anticorpo específico de doador (2,605 versus 202 mpi, p < 0,001), mas 3/8 pacientes evoluíram para perda do enxerto, conferindo sobrevida do enxerto de 62,5% (p < 0,001). **Conclusões:** O uso rotineiro da pesquisa de C4d e anticorpo doador específico aumentou a incidência de rejeição aguda. A rejeição aguda mediada por anticorpo apresentou perfil clínico e resposta terapêutica diferentes da rejeição aguda celular, conferindo-lhe pior prognóstico e pior resposta terapêutica.

**Descritores:** Rejeição de enxerto/diagnóstico; Rejeição de enxerto/terapia; Antígenos HLA; Transplante de rim; Plasmapherese

**INTRODUCTION**

After renal transplantation, insults of various natures may involve the graft and have a negative impact on long-term survival\(^1\). With the introduction of new protocols of immunosuppression, the incidence of acute rejection (AR) dropped drastically, but it is still an immunological event of extreme clinical relevance\(^2\). The immune response against the transplanted organ may be mediated by T-cells or by humoral response, triggered by antibodies that are specific against donor (DSA) beside other mechanisms that are yet not completely elucidated\(^3\). In the context of AR, there are important pathophysiological differences between acute cellular rejection (ACR) and the humoral rejection, more appropriately named acute antibody-mediated rejection (AMR). While the renal lesion in ACR is caused by an infiltrate of T-cells in the renal tissue, preferentially in the tubules, i.e., tubulitis\(^4\), AMR occurs after the recognition of an alloantigen for a DSA, with consequential activation of the classic complement pathway, leading to capillaritis, namely in peritubular capillaries, vasculitis, or fibrinoid necrosis\(^5\). Different from what happens in ACR, the diagnosis of AMR does not depend on optic microscopy, and there is a need for resources such as investigation of complement activation in the renal tissue, as well as research of circulating DSA. For this reason, the true magnitude of the problem is unknown, and it is speculated that there is an underestimated incidence in previously published data. Studies with various methodologies have demonstrated an incidence of about 8% for AMR, with conflicting results as to its impact on graft survival\(^\(3,6\)\).

Recently defined criteria for the diagnosis of AMR are the presence of graft dysfunction associated with characteristic histological changes, presence of diffuse deposits of C4d in renal tissue, verified with immunofluorescence or immunohistochemistry, and the demonstration of circulating DSA\(^4\). Over the last years, the use of more accurate techniques has been described for the detection of DSA, even at low titers, and it is possible to identify the intensity of each specificity, which is valuable for therapeutic follow-up\(^7\). Moreover, the introduction of C4d research in the routine of renal graft biopsies\(^8\) enabled each AMR to emerge as a new problem in renal transplantation. C4d is a marker of the classic complement activation pathway and its deposit in peritubular capillaries has been described as an AMR-specific finding\(^9\). There is a close correlation between the presence of C4d in the tissue and the detection of circulating DSA, with 95% sensitivity and 96% specificity when DSA is analyzed in C4d-positive patients\(^10\). In this way, the use of both techniques is fundamental for diagnosis as much as for therapeutic accompaniment of patients with AMR.

To date, there are still many questions as to the true impact of AMR on graft survival, just as the best therapeutic strategy is also not yet well established\(^6\). Recent studies have demonstrated that, despite treatment with plasmapheresis and immunoglobulin, and more recently, the use of the anti-CD20 antibody, AMR substantially increases the risk of renal graft loss\(^3\). The greatest efforts have been concentrated on the identification of patients at a high risk of developing AMR. The occurrence of AMR requires an immunologic memory against the alloantigen of the donor or it reflects the presence of low titers of antibodies, which are not detected by conventional cross-matching methods\(^3,4,6\). The use of more sensitive methods for cross-matching tests, such a flow cytometry\(^7\), as well as pre-transplant investigation of DSA in low titers may have a positive impact on the choice of donors, type of immunosuppression, and the best surveillance strategy after the transplant.

**OBJECTIVE**

To evaluate the incidence of AMR after the establishment of routine C4d research in transplanted...
kidney biopsies and DSA investigation by Luminex; to compare the characteristics of AMR relative to ACR, especially regarding therapeutic response and, finally, to assess the impact on the incidence of AMR after using cross-matching by flow cytometry and pre-transplant DSA investigation in patients with a history of prior exposure to alloantigens.

METHODS
A prospective analysis was carried out, including all the kidney transplants performed at the Kidney Transplantation Unit of the Hospital Israelita Albert Einstein between January 2007 and December 2008. During this period, 124 transplants were performed, 63 of them with a live donor and 61 with a deceased donor. The patients were assorted according to the occurrence of AR as ACR or AMR, although for statistical purposes AR episodes were considered. Renal biopsy was indicated in the following situations: 1- increased serum creatinine in patients with stable levels; 2- patients with delayed graft function (DGF) with a duration greater than one week after the transplant if the donor was deceased; and 3 - patients with DGF between the second and fourth postoperative day if the donor was alive. DGF was defined as the need for dialysis during the first week after transplantation.

Immunosuppression protocols
Immunosuppression of patients who received a live donor kidney consisted of 1.0 g methylprednisolone intraoperatively, followed by the introduction of a calcineurin inhibitor (cyclosporine for HLA-identicals and tacrolimus for the others), mycophenolate sodium (1,440 mg a day), and prednisone (0.5 mg/kg/day) as of the first postoperative day. Patients who received kidneys from deceased donors were induced with an intraoperative 1.0 mg/kg dose of thymoglobulin, followed by sequential doses, as per the CD3+ cell count, up to a total of four doses. The dose was administered only when the CD3+ count was greater than 20 cells/mm³. Mycophenolate sodium (720 mg a day) was introduced during the first postoperative day. Tacrolimus completed the immunosuppression of these patients, as it was introduced after the last dose of thymoglobulin, commencing when the CD3+ count was greater than 20 cells/mm³. The mycophenolate dose was doubled after the last dose of thymoglobulin.

In both types of patients, the dose of prednisone was progressively reduced, reaching 5 mg a day at the sixth month. The doses of mycophenolate were adjusted according to the presence of side effects – leucopenia or diarrhea.

Renal biopsy
All renal biopsies were analyzed using staining with hematoxylin-eosin, Masson trichromium, Schiff periodic acid, and silver impregnation. Acute cellular rejection was diagnosed and classified according to Banff’s criteria[11]. Suspect histological findings for AMR were: the presence of acute tubular necrosis, presence of inflammatory cells in the lumen of peritubular capillaries (capillaritis in peritubular capillaries), fibrinoid necrosis in vessel walls, or thrombi. In each biopsy, one of the tissue fragments was submitted to investigation of the complement factor C4d using immunofluorescence. The presence of deposits of C4d in more than 50% of the peritubular capillaries of the sample was considered indicative of a rejection process mediated by antibodies. All patients that proved C4d-positive with the characteristics described above were submitted to investigation of donor-specific antibodies. We considered the following set of alterations conclusive for the diagnosis of AMR: typical histological picture, diffuse deposits of C4d, and the presence of DSA. Only one patient was diagnosed with AMR without the detection of an anti-donor circulating antibody. Nevertheless, the histological picture showed fibrinoid necrosis on an arteriole wall and the immunofluorescence test showed C4d deposits in peritubular capillaries in 100% of the sample.

Cross-matching, lymphocyte panel reactive activity, and investigation of anti-donor antibodies
Pre-transplantation cross-matching was performed with the complement-dependent cytotoxicity technique with the addition of human anti-globulin (CDC-AHG), and in cases of a live donor for hypersensitized receptors, there was complementation with flow cytometry. In cases of transplants carried out with a deceased donor, the cross-matching was performed by the central unit for transplants of the state of São Paulo; in patients with a live donor, it was done at the Histocompatibility Unit of Hospital Israelita Albert Einstein during the week preceding the transplant. The panel reactive activity (PRA) was also performed by the central unit for transplants using ELISA methodology. In C4d-positive patients submitted to renal biopsy, the investigation of antibodies against donor HLA was performed using the LABScreen™ technique, with determination of specificities as per the Luminex platform, employing reagents Single Antigen, One Lambda. C4d-positive patients with no detected antibodies specific against donor HLA, we searched for anti-MIC A and B antibodies. We considered a patient positive for the presence of antibodies when the fluorescence of the reaction was greater than 500 mfi.
As of June 2008, the investigation of specific antibodies against HLA antigens became a routine procedure before the transplant, utilizing Luminex in patients previously sensitized by transfusions, gestations, or retransplantations. For transplants with live donors, it was possible to detect specific antibodies against the donor. In the case of patients on the waiting list, we used PRA to determine which HLA antigens were permissible for the transplant, with the possibility of performing virtual cross-matching.

Treatment of acute rejection
For treatment, ACR was classified as early when it occurred within the first two months after the transplant, or late, when it appeared after this period. Banff IA or IB patients with early or late ACR were treated with methylprednisolone pulse doses of 10 mg/kg of body weight, never surpassing 1.0 g per dose, during three to five days. Early Banff IIA rejection was initially treated with the same dose of corticosteroid, but for five days. Early IIB or III ACR cases or other episodes of ACR that did not respond to treatment with corticosteroids were treated with thymoglobulin. AMR cases were treated with methylprednisolone pulses of 10 mg/kg of body weight for three days in association with plasmapheresis sessions every other day. At the end of every third plasmapheresis session, PRA was performed to detect the fluorescence of each antibody specificity. Additionally, a 400 mg/kg dose on intravenous immunoglobulin was administered after the last plasmapheresis. Treatment for AMR was interrupted after renal function improvement, when the specific antibody became negative or after histological improvement. Patients presenting with concomitant AMR and ACR also received thymoglobulin.

Statistical analysis
Outcomes assessed were: renal function at the time of diagnosis of rejection, after treatment, and at the end of follow-up, besides the impact of the acute rejection episode on the rate of graft losses and survival. Numerical variables were summarized as means and standard deviation, and in cases of non-normal distribution, the median was added in parenthesis. Results were compared using Student’s t-test. Categorical variables were summarized as percentages and were compared by chi-Square or by Fischer’s exact test, as per the N in each comparison. Renal graft survival was calculated using Kaplan-Meier’s method. Calculations were performed with SPSS for Windows software, while the graphics were constructed using Prisma. Statistical significance was defined by a value of p inferior to 0.05, with a 95% confidence interval.

RESULTS
Demographic data
Of the 124 transplanted patients, three were excluded from this analysis because they evolved to obit during the immediate postoperative period. All three of them received grafts from deceased donors; one patient experienced disseminated intravascular coagulopathy and the other two went into refractory shock after anesthetic induction. Among the 121 individuals, 63 received transplants from live donors (52%) and 58 from deceased donors (48%). Follow-up time was 14.7 ± 7.7 months. The demographic characteristics of the analyzed population are listed on table 1.

Prevalence of acute rejection
The prevalence and number of episodes of acute rejection (AR) were analyzed. General prevalence of AR was 18.8% (24/121). Twenty-six episodes were diagnosed. Two patients presented with both histological types, but in biopsies from different time points: one patient displayed ACR on the fourth day of transplantation and one episode of AMR diagnosed on the 17th day; the other had AMR on the 18th day and ACR on the 49th day. There were eight episodes of AMR, with a prevalence of 6.6% (8/121), and 18 of ACR, with a prevalence of 14.9% (18/121). Among the patients who received a live donor graft, prevalence of AR was 20.6% (13/63), with 12 episodes of ACR (19%) and 2 episodes of AMR (3.2%). Of those who received a deceased donor graft, the prevalence of AR was 18.9% (11/58), and there were six episodes of each histological type (10.3%).

The ACR cases occurred at a mean time of 59.9 ± 84.4 days [median = 29(3-267)], in which ten episodes...
Diagnosis and treatment of acute antibody-mediated rejection in renal transplant: the role of C4d and donor-specific antibody identification

(55.5%) appeared before hospital discharge, three between the time of discharge and 60 days after the transplant (16.7%), and five after 60 days post-transplantation (27.8%) (Figure 1). According to Banff criteria, two patients were classified as displaying borderline criteria and were treated as ACR (11%), seven had ACR IA (39%), five displayed type IB (28%), one showed IIA (5%), and there were three patients with type IIB (17%) (Figure 2). There were no episodes of ACR III. The AMR episodes occurred, on average, at 12.5 ± 5.4 days [median = 11(6-21)], which is earlier than the ACR cases, but this difference did not reach statistical significance (p = 0.13). For confirmation of the diagnosis of AMR, every time the C4d investigation was positive, tests for antibodies specific for the donor HLA were carried out. Table 2 details the specificities for donor HLA of each patient that evolved with AMR. Among these eight patients, one did not have anti-HLA antibodies, although the presence of the anti-MICA antibody was detected (patient 5), and in another individual who presented with fibrinoid necrosis and positive C4d in 100% of the sample, we failed to identify Anti-HLA or Anti-MICA, but the patient was considered as having AMR, according to the other criteria (patient 1). All eight patients had acute tubular necrosis seen by histological testing, and this was the only alteration noted in three patients (patients 3, 4, and 7 – from table 2). Capillaritis in peritubular capillaries was observed in four patients (patients 1, 5, 6 and 8) and glomerulitis in another three (patients 1, 2 and 6). Finally, the criterion for diagnosis of vascular rejection was observed in two patients (patients 1 and 5). C4d testing was positive in 100% of the sample in four patients (patients 1, 2, 6 and 7) and in 50% of the sample in the other four patients (patients 3, 4, 5 and 8).

Figure 3 demonstrates the histology of the kidney biopsy of a patient presenting with AMR, stained with HE, showing glomerulitis, capillaritis in peritubular capillaries and the presence of C4d in 100% of the peritubular capillaries (patient 7). As of June 2008, when cross-matching using flow cytometry and pre-transplant DSA testing for live donors with a history of sensitization or the performance of virtual cross-matching through the determination of permissible antigens for receptors listed for a deceased donor were implemented, no new cases of AMR were observed.

Table 2. HLA receptor, donor and DSA

<table>
<thead>
<tr>
<th>Patients</th>
<th>A</th>
<th>B</th>
<th>Dr A</th>
<th>A</th>
<th>B</th>
<th>Dr A</th>
<th>A</th>
<th>B</th>
<th>Dr</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>29</td>
<td>45</td>
<td>57</td>
<td>11</td>
<td>15</td>
<td>1</td>
<td>74</td>
<td>40</td>
<td>52</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>30</td>
<td>8</td>
<td>18</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>33</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-</td>
<td>39</td>
<td>40</td>
<td>4</td>
<td>14</td>
<td>2</td>
<td>31</td>
<td>15</td>
<td>39</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>31</td>
<td>35</td>
<td>81</td>
<td>14</td>
<td>15</td>
<td>31</td>
<td>69</td>
<td>35</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>-</td>
<td>42</td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>35</td>
<td>53</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>23</td>
<td>44</td>
<td>50</td>
<td>7</td>
<td>15</td>
<td>23</td>
<td>66</td>
<td>44</td>
<td>58</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-</td>
<td>37</td>
<td>57</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>-</td>
<td>44</td>
<td>-</td>
<td>4</td>
<td>15</td>
<td>3</td>
<td>31</td>
<td>41</td>
<td>44</td>
</tr>
</tbody>
</table>

HLA A, B and Dr: HLA locus in each patient; MIC: specific antibody against donor endothelium.
Comparison between ACR and AMR

When the demographic characteristics (Table 1) of patients presenting with ACR and AMR were analyzed, it was observed that among the AMR patients, when compared to the ACR patients, 75% of the patients with AMR were females versus 29.9% (p = 0.05); 75% received kidneys from deceased donors versus 33.3% (p = 0.09), and had a longer time on dialysis (87.7 ± 51.2 versus 47.4 ± 37.3 months, p = 0.03). The variables related to sensitization were also significantly more frequent in patients with AMR: the number of transfusions was three times higher among these patients (p = 0.02); PRA was 28.0 ± 37.4%, versus 4.8 ± 13.1% (p = 0.03), and they were more frequently retransplanted (50 versus 5.6%, p = 0.02). After transplantation, DGF occurred in all patients with AMR and in half of those with ACR (p = 0.02). As to renal function, serum creatinine at the time of diagnosis was significantly worse among patients with AMR (6.6 ± 2.1 versus 3.1 ± 1.6 mg/dl, p < 0.001).

Treatment of AR

For each DSA, we measured fluorescence of the antibody using LABScreen™ (in mfi) for therapeutic follow-up purposes. All patients with AMR received a methylprednisolone pulse, and only one (patient 4) responded adequately, with recovery of renal function. The other seven received plasmapheresis, with an average of 7.3 ± 2.2 sessions, followed by 1.9 ± 1.0 doses of intravenous immunoglobulin. Before treatment, patients who displayed anti-HLA antibodies had a fluorescence of 2,605.7 ± 2,817.3 mfi (1,494) and after treatment it was 202.4 ± 145.0 mfi (262), with p < 0.001 (Figure 4), demonstrating that this strategy was highly effective in reducing DSA titers. There was no adverse event related to plasmapheresis. One patient experienced an anaphylactic response during the infusion of immunoglobulin, which was reversed with clinical support measures. All the patients with ACR had their graft function restored. There was improvement of creatinine in both groups, but the patients with AMR maintained significantly higher levels (4.3 ± 3.6 versus 2.2 ± 1.0 mg/dl, p = 0.03). We also compared the last creatinine value in follow-up of these patients, and similarly, patients with AMR showed worse graft function: 3.8 ± 3.6 versus 1.6 ± 0.6, p = 0.01 (Figure 5).
Diagnosis and treatment of acute antibody-mediated rejection in renal transplant: the role of C4d and donor-specific antibody identification

Outcomes

In this population of 121 patients, four died (1.9%), two of them due to sepsis, one due to an intra-abdominal sarcoma, and another by subarachnoid hemorrhage. All of them died with their kidneys functioning. One had presented with ACR (deceased due to a sarcoma) and another, with AMR (deceased due to sepsis). Besides these, three other patients died soon after the surgical procedure, as previously reported, with a total sum of deaths of 5.6%. In the study population, there were eight losses, with a prevalence of 6.6%. There were three graft losses among the patients with AMR and none among those with ACR; therefore AR is the cause of 37.5% of all losses. Graft survival (Figure 6) of the total study population was 93.4% at the end of 1 year, i.e., 100% among patients with ACR and 62.5% among those with AMR (p < 0.001).

DISCUSSION

Acute rejection is one of the main insults that can occur after renal transplantation\(^{(1)}\), and it is classified, as per the immune mechanism involved, in cell-mediated rejection or antibody-mediated rejection\(^{(4)}\). During the 1970's and 1980's, at least one AR episode occurred in more than 50% of transplanted patients, and the main cause was graft loss, with serious limitations to long-term survival\(^{(12)}\). With more recent immunosuppression protocols, it has been possible to reduce the incidence of AR, reaching rates of 13 to 15% in some studies\(^{(13-14)}\). In this study, we performed a prospective analysis using as routine the early indication of a biopsy to assess patients with DGF or with alterations in renal graft function, C4d testing in tissues, and techniques sensitive enough to identify donor-specific antibodies. We noted an AR incidence of 18.8%, with 30% of the acute rejection episodes mediated by antibodies \(8/26\). In a prior study that evaluated a cohort of kidney transplant patients who received organs from deceased donors at our institution, before initiating the protocols herein described, the AR incidence was at the rate of 9%\(^{(15)}\). The increase in rate of AR demonstrated in this study occurred due to the increase in number of diagnoses of AMR. Two important studies designed with the objective of defining the profile of AR in renal transplantation, as well as the incidence of AMR, followed a frequency of AR at the rate of 20 to 30%, with a clear predominance of the cellular type\(^{(3,6)}\). The rate of AMR has been described as between 3 and 8%\(^{(16)}\). Rocha et al., in a retrospective study that assessed the incidence of AMR in patients with C4d testing and DSA, observed an incidence of 5.6%\(^{(6)}\), while Crespo et al., with similar methodology, noted a rate of 7.7%\(^{(3)}\). After implementing routine C4d testing in all biopsies and DSA testing in C4d-positive patients, the incidence of AMR was 6.6%. Investigation of C4d and DSA are indispensable weapons for the identification of AMR\(^{(4,17-18)}\). The criteria that define the diagnosis of AMR are typical histological lesions, positive C4d in renal tissue, and circulating DSA. The characteristic histological types vary from acute tubular necrosis to fibrinoid necrosis\(^{(19)}\), and histology using optic microscopy is not capable of predicting AMR\(^{(20)}\). Therefore, the identification of AMR depends on standardization of these two routines in the clinical practice of renal transplantation.

We also assessed the primary clinical characteristics of patients that presented with AMR and compared them to those who had ACR. The differences between the two types of rejection confirmed data previously published, demonstrating that they are two entities with completely distinct immune and clinical natures\(^{(6,19)}\). From the clinical point of view, AMR occurs earlier than ACR, generally before hospital discharge\(^{(6)}\). Among our patients, all

---

Figure 5. Kidney function according to rejection type

Figure 6. Graft survival according to acute rejection type

\(\text{Cr1: creatinine upon diagnosis of acute rejection (6.6 ± 2.1 versus 3.2 ± 1.6 mg/dl, p < 0.001); Cr2: creatinine after treatment (4.3 ± 3.7 versus 2.2 ± 1.0 mg/dl, p = 0.031); Cr3: last creatinine of follow-up (3.8 ± 3.6 versus 1.5 ± 0.5 mg/dl, p = 0.014)}\)
experienced AMR before the third week post-transplant. Despite not having evaluated an explanation for this fact, we speculated that most of the patients with AMR have an immunologic memory for alloantigens and a re-exposure after the graft implantation is capable of triggering a humoral response. Even if patients do not display previously produced circulating DSA, it is possible that prior sensitization to alloantigens different from the donor renders them “high-responders”\(^3\). According to this theory, we noted that patients with AMR were more frequently re-transplanted, had a greater historic PRA, as well as a history of more frequent transfusions. Added to this is the finding that among them there was a predominance of female individuals and only one of these patients had not been previously pregnant. Therefore, a humoral immunologic memory response has a vital role in the pathogenesis of AAMR, which could create a state of “humoral alloreactivity”\(^{21-23}\), facilitating a donor-specific humoral response. We also observed that there was a predominance of deceased donors and a greater incidence of DGF in patients with AMR. This finding is consistent with the idea that the lesion of ischemia and reperfusion increases the immunogenicity of the graft, with an increased expression of MHC molecules after the initial insult\(^23\).

We noted that, at the time of diagnosis, serum creatinine of patients with ACR was significantly lower than that of patients with AMR. From a clinical point of view, a possible explanation is the greater frequency of deceased donors and of DGF among patients with AMR. Another important difference between the two types of rejection is the histological presentation. ACR is marked by the infiltrate of inflammatory cells in renal tubules, requiring the rupture of the tubular basement membrane, while despite not being the most characteristic trait, the most common presentation of AMR is acute tubular necrosis\(^3,20\). Half of the patients in our population displayed DGF and the histological translation of DGF is acute tubular necrosis. It means that cases of AMR may not be detected if the investigation of C4d in renal tissue is not used. In our study, of the eight patients who presented with AMR, all had ATN observed in histology, and in three patients, this was the only histological finding.

As previously demonstrated, the therapeutic response is substantially better among patients with ACR, with no graft loss due to AR among them besides normalization in function of the graft after the treatment. In spite of progressive reductions in rates of acute rejection, its occurrence is still an important marker of the long-term prognosis of the graft\(^1\), especially in early episodes, which are those that appear before 60 days post-transplantation. Early rejection reduces 20 to 30% in the expected survival for the graft\(^24\) and even when there is complete recovery of renal function after treatment of the rejection, a 10% reduction is expected. Most of the studies that assessed this impact did not evaluate the differences in the subtypes of AR. We observed that 72% of the episodes of ACR and all of the AMR episodes were early (less than 60 days), thus predominating before hospital discharge. Different from previously published data, there was no graft loss during the first year among patients with ACR. One possible explanation for this finding might be the predominance of light stages of AR: about 70% among criteria for borderlines and Banff I (Figure 2). Despite the intense immunosuppressive treatment, one-year survival of the renal graft among patients with AMR is 50\%\(^{25-26}\). Utilizing plasmapheresis and immunoglobulin, some studies have demonstrated a one-year survival of 80\%\(^{3,6}\). In our study, more than one third of the patients had lost the graft before one year post-transplantation. Among the three losses, two occurred due to rejection and in a third, even with histological improvement after treatment, there was no functional recovery. These three losses were related to more severe histological lesion, fibrinoid necrosis, and a longer time to diagnosis. One other patient with AMR died after one year of the transplant due to sepsis, but with a functioning kidney. Among the eight patients treated, we considered that five responded adequately, with recovery of the graft function. Among these patients, serum creatinine levels were similar (1.3 ± 0.4 mg/dl) to those of patients with ACR, demonstrating that early diagnosis, when more severe histological lesions have not yet been established, may change the response to treatment. Most institutions have used plasmapheresis with intravenous immunoglobulin for the treatment of AMR\(^3,6,26\). This combination was very effective in reducing circulating DSAs (Figure 5). The mechanism of action of intravenous immunoglobulin is not well determined yet, but it may be related to opsonization of antibodies, facilitating removal by the reticuloendothelial system, as well as immunomodulation. More recently, the use of rituximab has been proposed for its treatment\(^27\), but there are no conclusive data and its use with this indication is not yet authorized by health authorities in our country.

All our cases of AMR occurred up until May 2008. As of this period, we have used investigation of anti-HLA antibodies utilizing LABScreen\textsuperscript{™}, with determination of specificities with the Luminex platform (reagents Single Antigen, One Lambda), besides performing cross-matching by flow cytometry for patients with deceased donors when the receptors had a history of prior sensitization. These techniques proved to have a better specificity profile; nevertheless, there is a need for more studies in order to establish the best clinical practice in
the case of hypersensitized patients, which are the ones at greater risk for AMR, as demonstrated: history of transfusions, retransplantations and pregnancy.

CONCLUSIONS

In this prospective study, the incidence of acute rejection was determined, as well as the histological and clinical profile of cell-mediated and antibody-mediated rejection types. It was demonstrated that AMR is an early event in transplantation, related to patients with a prior history of sensitization to HLA antigens, and the prognosis is closely related to the type of histological lesion present at diagnosis. The treatment with plasmapheresis and intravenous immunoglobulin was very effective in reducing the levels of circulating DSA, besides improving the histology and renal function of most of the patients.

REFERENCES