

Successful live donor kidney transplant in a hypersensitised patient with positive cross-match. A case report and a review of the effectiveness of desensitisation protocols.

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■ ABSTRACT

We present a case of a hypersensitised end stage renal disease patient with impossibility of peritoneal dialysis and exhaustion of vascular access for haemodialysis, who presented a positive cross-match to her potential living related donors. A desensitisation protocol was applied and a renal transplant was successfully performed with the kidney from the living donor. The protocol was based on high-dose unspecific immunoglobulin (IV Ig 2g/Kg), after which the patient converted to a negative cross-match and was successfully transplanted. Induction immunosuppression consisted of ATG, tacrolimus, mycophenolate mofetil and steroids. The transplant course was complicated by an antibody mediated acute rejection (AR), treated with bolus methylprednisolone, plasmapheresis and IV Ig (1g/Kg). There was complete recovery with no further AR episodes. After fifteen months of follow-up the patient was in good health with stable renal function (creatinine 1.1 mg/dl; creatinine clearance by the Cockcroft-Gault equation 74.6 ml/min).

This desensitisation protocol was successful in converting from positive to negative flow-cytometry cross-

match. Renal transplant was successfully performed after conversion.

Key-Words:

Intravenous immunoglobulin; kidney transplant; positive cross-match; sensitised patient; desensitisation protocol.

■ INTRODUCTION

While kidney transplantation is the therapy of choice for patients with end stage renal failure, the waiting list for kidney transplantation lengthens dramatically year by year. The discrepancy between demand and supply propels the search for strategies to expand the pool of donors. The shortage of deceased donors has led to the increased use of living donors. Recently the rate of renal transplant (RT) from living donors has increased significantly all over the world and Portugal is no exception. Despite this increment in live donation, many recipients with otherwise suitable donors are relegated to the deceased donor waiting list because they have a positive cross-match with their potential living donors. Patients high-

ly pre-sensitised, with a last panel reactive antibodies (PRA) $\geq 50\%$, are less likely to be transplanted and will spend long years on the waiting list. Human Leukocyte Antigen (HLA) sensitisations result from exposure to disparate HLA antigens from other individuals and are acquired through prior transplantation, blood transfusions and pregnancy. Over half of these broadly sensitised patients are women. These patients are difficult to transplant and RT is delayed for years due to repetitive positive cross-matches^{1,2}. Once these patients finally undergo transplantation the long-term graft survival is significantly reduced through higher frequency of acute rejection (AR) and an increase in chronic graft dysfunction due to chronic rejection, leading ultimately to graft loss².

Renal transplant in hypersensitised patients is problematic. The difficulty in getting a compatible deceased donor, particularly when associated with the impossibility of peritoneal dialysis and the exhaustion of vascular access for haemodialysis, seriously limits the vital prognosis of these patients. The possibility of living donor RT is often conditioned by immunological incompatibility, which translates as a positive cross-match. Previously these patients were excluded without further workup. Innovative strategies have been developed in recent years by several groups in order to carry out RT across this barrier. Recently described desensitisation protocols with unspecific intravenous immune globulin (IV Ig) with or without plasmapheresis suggest a high effectiveness which can modify the prognosis of patients with a positive cross-match. Patient and graft survival are excellent despite a notoriously high rate of acute humoral rejection. In this way contra-indication related to the presence of pre-formed HLA antibodies has become relative^{1,2}.

Our centre is transplanting increasing numbers of patients with living related donors and has been trying to further expand this valuable donor source. The living donor kidney transplant represents the ideal setting for the application of desensitisation protocols.

■ CASE REPORT

We report a 34-year-old Caucasian female with a past history of frequent urinary tract infections in infancy. Her radiological exam presented features of pyelonephritis in the right kidney and pyelocaliceal

duplicity in the left kidney, suggesting reflux nephropathy. During her first pregnancy in 1994 she presented pre-eclampsia that resolved with delivery. In 1997 she presented painless ulcers in the mouth, malar rash, pancytopenia, arthritis, haematuria and mild renal insufficiency, together with serological markers suggesting the diagnosis of systemic lupus erythematosus. Renal biopsy disclosed diffuse proliferative lupus nephritis (active diffuse global glomerulonephritis involving $\geq 50\%$ of all glomeruli, with diffuse subendothelial immune deposits) and membranous nephritis (light microscopy and immunofluorescence revealed global subepithelial immune deposits) – Class IV+V. She underwent six cycles of pulse cyclophosphamide therapy and steroids without significant improvement, maintaining nephrotic syndrome and progressive renal failure. Haemodialysis by an arteriovenous fistula was started in April 1999. She was evaluated for a kidney transplant and placed on the active waiting list in 1999. Haemodialysis course was complicated by recurrent arteriovenous fistula thrombosis with need for placement of multiple central venous catheters also complicated by repetitive infections. Antiphospholipid antibodies, namely antibodies to the phospholipid cardiolipin and the plasma protein β_2 glycoprotein I, were negative. She was then transferred to continuous ambulatory peritoneal dialysis (CAPD) in March 2001. During the CAPD period she had multiple peritonitis episodes with gradual ultrafiltration loss. In November 2002 loss of renal residual function and ultrafiltration failure caused her transfer to automated peritoneal dialysis, without success. Return to haemodialysis by a tunneled central venous catheter became necessary in December 2002. Despite being in urgent degree for RT, a compatible donor was not found. She was hypersensitised with a PRA of 98%. At that time two potential living donors were evaluated. They were suitable for living donation but were refused due to positive cross-match (April 2002: positive cross-match with the mother; May 2002: positive cross-match with the father).

Haemodialysis treatment was complicated by recurrent central venous catheter infections and obstructions. In September 2003 a polytetrafluoroethylene (PTFE) prosthetic graft was placed in the right arm as vascular access for haemodialysis. In April 2004 a venous stenosis of the prosthetic graft needing surgical revision was detected. A re-stenosis with venous hypertension syndrome occurred, leading to a stent

placement. During the dialysis period she also presented lupus flares with cutaneous and haematological involvements. She required multiple blood transfusions and was treated with steroids. In April 2004 she presented severe metrorrhagia requiring frequent transfusional support without recovery with medical treatment. Hysterectomy was performed and complicated with a pelvic abscess, with need for reoperation, followed by difficult wound healing. During this period, she developed Wernicke encephalopathy that recovered with high doses of thiamine. She also developed a serious cutaneous pseudoporphyria tarda and intestinal sub-occlusion episodes treated with medical and conservative measures that placed the patient in intense suffering and depressive syndrome.

In the face of the vascular situation for haemodialysis, exhaustion of peritoneal dialysis and the hypersensitisation problem (peak PRA: 98%; current PRA: 87%; alloantibodies class IgG, targeting HLA antigens class I and II), a new attempt at evaluation for RT with living donor was made. Cross-match was positive with both parents. It was decided to use a desensitisation protocol.

The desensitisation protocol consisted of high dose unspecific IV Ig (2g/Kg), after which cross-match was repeated. Cross-match used was antihuman globulin-modified complement-dependent cytotoxicity (AHG-CDC) and flow-cytometry (FC).

The first cycle of IV Ig was on 06 April 2005, after which a negative cross-match for AHG-CDC for T and B cells from her mother was obtained, but the classic CDC was positive for B cells. It was decided to initiate immunosuppression with tacrolimus (FK) plus mycophenolate mofetil (MMF) on 28 April 2005 and a second course of high dose IV Ig was repeated one month later on 2 May 2005. Cross-match then became negative for AHG-CDC, classic CDC and FC for B and T cells. The immunological study at that time revealed ANA 1/640 and low C3 and C4. Platelet count was 72000/ μ l. On 3 May 2005 the renal transplant was made with her mother's kidney (2 mismatches – donor HLA A 01,00; B 07,08; DR 01,03 and receptor HLA A 01,03; B 07,27; DR 01,15; current PRA: 51%). Immunosuppression consisted of FK with target blood levels of 12 - 15 ng/ml plus MMF from day -6 and induction with ATG (3 to 5 mg/kg during the first 10 days post RT) and steroids.

No immediate complications occurred and she had immediate graft function. On the eighth day post transplantation creatinine was 0.7 mg/dl. On the 9th day there was a sudden decrease in the urinary flow, fever and allograft pain, together with a rise in serum creatinine (>25%; 1.5 mg/dl). Acute rejection was suspected and an ultrasound guided renal transplant biopsy performed. Renal biopsy revealed no cellular infiltration or alterations suggestive of vascular AR and mild acute tubular necrosis (the anti-C4d staining was unavailable at that point). Repeated cross-match with the mother's serum revealed as positive. Antibody mediated AR was suspected and treated with bolus methylprednisolone and plasmapheresis, followed by IV Ig (1g/Kg). There was complete recovery of the symptoms and serum creatinine. There were no more episodes of AR and the patient was discharged on the 17th day with a creatinine of 0.98 mg/dl. She received valganciclovir and cotrimoxazole for cytomegalovirus and *Pneumocystis Carinii* prophylaxis for 3 months. IV Ig was repeated, as defined in the protocol on days + 21 and + 120. In August 2005 she presented positive class I and class II alloantibodies (luminex technique) and a PRA of 93%. During the fifteen months of follow-up the patient remained asymptomatic, with mild hypertension controlled with ramipril 2.5 mg/day, without lupus flares and with disappearance of the pseudoporphyria lesions. She presents no abnormalities in blood chemistry, has stable immunological study and good renal function with serum creatinine 1.1 mg/dl, creatinine clearance (by the Cockcroft-Gault equation) 74.6 ml/min. Current immunosuppression is prednisolone 10 mg/day, MMF 1.5 g/day and FK 10 mg/day.

■ DISCUSSION

Patients with preformed HLA antibodies or ABO-blood group incompatible with the intended donor have in recent years been successfully transplanted. Reports of successful RT across these barriers using various desensitisation protocols has stimulated interest in using immunological incompatible grafts³. There are currently two types of desensitisation protocols, with some variations, to successfully transplant these broadly sensitised patients. Some researchers have used high dose IV Ig while others have used plasmapheresis and low dose IV Ig with similar results. Some also used rituximab and splenectomy. At the moment

there are no comparative studies that could clarify which is the more effective protocol^{1,2}.

Intravenous immune globulin products are known to have powerful immunomodulatory effects on numerous autoimmune and inflammatory disorders. The proposed mechanisms of action are multiple and complex and include neutralisation of circulating antibodies through the presence of IgG anti-idiotypic antibodies, inhibition of cytokine gene activation, inhibition of cytokine secretion and anti-cytokine activity, reduction in antibody formation, inhibition of B and T cell proliferation, anti-CD4 activity, inhibition of complement dependent injury and other immunomodulatory actions. All these mechanisms may be responsible for inducing a profound and sustained decrease in the titres of anti-HLA antibodies that are important in modulating the immune response^{1,2,4-7}. In a study investigating the mechanism of the action of IV Ig on anti-HLA antibodies, the authors found that IV Ig do not appear to bind lymphocytes but inhibit the binding of anti-HLA antibodies to the cells in a dose-dependent manner which argues in favour of high dose desensitisation protocols².

Desensitisation protocols with high dose IV Ig (without plasmapheresis) has been carried out over the last few years, yielding varying results. The optimal dose and frequency of administration, however, remain unclear. Glotz *et al*⁶ reported on 15 patients (with either a PRA of > 50% or with a positive T cell cross-match to their potential living donor) treated with high dose IV Ig (2g/kg over 48 hours) given every 4-weeks for a total of 3 doses. Immunosuppressive regimen consisted of MMF, steroids and thymoglobulin 10 days, associating FK. The IV Ig was given at the same dose on day 0 and 1 and also repeated on days 20-21 and 40-41 post RT. Thirteen (87%) patients were successfully desensitised and underwent RT. They lost 1 transplant due to thrombosis and 1 due to rejection. The post-transplant clinical course after 1 year of follow-up was uneventful for the remaining 11 patients without AR episodes⁶. This study raises concerns about adverse effects potentially associated with high dose IV Ig, such as fever, chills and severe headache and other less frequent but more severe side effects as anaphylaxis, severe thrombosis (deep venous thrombosis, central retinal vein occlusion, transplant renal vessel thrombosis) or nephrotoxicity⁶. In our patient we did not report any side effect of high dose IV Ig.

Another group⁷ reports their experience with 45 broadly sensitised patients (28 candidates for living donor RT; 15 candidates for deceased RT; and 2 candidates for heart transplantation). The potential living donor recipients underwent an initial *in vitro* cytotoxicity test (IV Ig was added to the cross-match test) to evaluate whether antibodies present in the IV Ig could inhibit or reduce cytotoxicity. If the *in vitro* test demonstrated inhibition, the recipient was given high dose IV Ig (2g/kg) usually in a single dose. Then, if the cross-match repeated immediately after the infusion became negative, the transplant was performed within 24 to 72 hours. An additional dose of IV Ig (2g/kg) was administered one month after transplantation. In the deceased donor candidates the PRA test was repeated in the presence of IV Ig. If the PRA was significantly decreased the patients received high dose IV Ig monthly for 4 months. In this series 42 patients underwent transplantation with graft survival rates at 24 months of 97.6% and 89.1%. They concluded that IV Ig inhibition of *in vitro* donor-specific cross-match predicted the *in vivo* responses to IV Ig, allowing efficient transplantation⁷.

High dose IV Ig was also used successfully to reverse established acute humoral rejection, as reported by Jordan *et al*⁸ who effectively reversed established acute humoral rejection in 10 transplant recipients. In a randomised study Casadei *et al*⁹ showed that high dose IV Ig had the same efficacy as OKT3, in 30 steroid resistant AR, rescuing 73% of the grafts without the side effects of OKT3.

In 2005 we initiated the application of a desensitisation protocol at our institution based on high dose IV Ig (2g/Kg) and this patient is the first case. This protocol is considered for hypersensitised patients with a long RT waiting time and an available living donor, found to have a positive cross-match by CDC (complement-dependent cytotoxicity). The protocol consists of immunosuppression with FK (trough blood levels of 12-15 ng/ml) and MMF (1g twice a day) from day -6 on. On day -1 high dose unspecific IV Ig (2g/Kg) is administered after the dialysis session. After this procedure cross-match is repeated for AGH-CDC and FC. If negative, RT is performed in the following 24 hours. If cross-match remains positive after the first dose of IV Ig, high dose IV Ig (2g/Kg) is repeated monthly for a maximal of 3 months. Cross-match is repeated after each IV Ig infusion. We used AHG-CDC because the addi-

tion of antihuman globulin increases the sensitivity of the test by inducing cross linking of any antibody present, thus increasing the likelihood of visualising cytotoxicity. Induction immunosuppression consists of ATG (3 to 5 mg/kg - 10 days) and steroids (intravenous methylprednisolone 500 mg started at the time of transplantation, and corticosteroids tapered to an oral dose of prednisone, 5 mg daily by 6 months post transplantation). IV Ig (2g/Kg) is repeated on days +21 and +120.

Other authors use repeated plasmapheresis or immunoabsorption techniques (to remove anti HLA-antibodies) coupled with low dose IV Ig (to prevent the rebound in antibody synthesis) with or without rituximab, in addition to immunosuppressive agents. The success rates of these protocols were variable and the costs and side effects not unremarkable^{1,2}. Montgomery *et al*¹⁰ first reported the successful treatment of 4 patients with a protocol which used a combination of plasmapheresis and low dose IV Ig to successfully transplant patients with positive cross-match with the potential donors. All patients presented acute humoral rejection. In this study the authors also demonstrated that this protocol could successfully reverse established refractory acute humoral rejection mediated by antibodies specific for donor HLA antigens. They were able to effectively reverse acute humoral rejection in 3 patients cross-match negative prior to RT but who developed donor specific antibodies at the time of diagnosis of acute humoral rejection¹⁰. A similar protocol was used by Schweitzer *et al*¹¹ that was able to transplant 11 of 15 cross-match positive candidates with the donor. All 11 recipients had low pre-treatment cross-match titres, however. Gloor *et al*¹² used a more aggressive protocol in 14 patients with a positive cytotoxic cross-match. In this study the protocol used not only plasmapheresis and IV Ig (100 mg/Kg) but also splenectomy and anti-CD-20 antibody (rituximab), an intensive regimen that incorporates interventions previously used for ABO-blood group incompatible RT. Reported success rate was 79% for allograft survival. The incidence of antibody mediated AR was 43% all reversible with plasmapheresis and steroids¹². Thielke *et al*¹³ described their experience with 16 potential recipients with a positive cross-match to the potential living donor. They used plasmapheresis and low-dose IV Ig (100 mg/Kg) every other day, starting 1 week before the presumable day of RT and continued every other day for a week post transplant. A single dose of

rituximab was also given to 6 patients at the beginning of treatment. RT was possible in 75% of the patients. Reported rate of AR was 41% (25% humoral), all successfully treated. Patient and graft survival at 1 year was 100%¹³. A further group used IV Ig and thymoglobulin immunosuppression for RT candidates with positive cross-match (FC positive for T and B cells) and reported a 88% allograft survival after an average follow up of 15 months¹⁴.

In analysing the results of the different protocols, the use of rituximab and splenectomy, although useful in the setting of ABO-blood group incompatibility, does not seem necessary for RT of cross-match positive patients.

Another drawback with desensitisation protocols may be the potential high risk of more frequent or aggressive infections, high rates of polyomavirus nephropathy and neoplasia¹. In the majority of the studies, however, a higher rate of infectious complications has not been verified^{10,13}.

Most series describe a high incidence of early acute humoral rejection despite a negative cross-match before RT^{2,7,13}. This outlines the high immunological risk and complexity of these patients. In our patient the clinical syndrome and sudden creatinine increase (over 25% of the baseline) on day 9 in the absence of any other explanation for allograft function deterioration raised the suspicion of AR. An ultrasound guided biopsy was obtained. Renal biopsy was not diagnostic and the C4d stain was unavailable to rule out humoral rejection. Repeated cross-match with the mother's serum revealed positive. Antibody mediated AR was suspected and treated with plasmapheresis (one plasma volume exchange using albumin replacement), bolus methylprednisolone (500mg/day for 3 days, followed by tapering dose of corticosteroids over the course of 1 week to the previous prednisolone dose) and IV Ig (1g/day) for 3 days. The patient presented complete recovery.

Anti-HLA antibodies may be detected using different techniques that vary in sensitivity and specificity. Different types of antibodies may be identified with varied clinical relevance. In such a way, while IgG antibodies detected by cross-match assays are habitually considered as true sensitisation, the rule of the IgM antibodies is not considered typical of true sensitisation and its value remains debatable. Antibod-

ies detected against T cells represent true anti-HLA sensitisation against class I antigens. On the other hand B cell positive cross-match, with T cell negative results, has been considered to represent antibodies against class II HLA with less clinical significance. Some authors, however, argue that this pattern of cross-match could mean low titres of pre-existing HLA class I antibodies, as B cells also express HLA class I antibodies. A current positive FC cross-match or a positive CDC or AHG-CDC cross-match with historic serum is associated with an intermediate risk for antibody mediated AR. In this context augmented immunosuppression may be required, as graft survival is worse in this patient population^{1,2,15-17}. Our patient presented a negative cross-match for AHG-CDC for T and B cells from the mother, but the classic CDC was positive for B cells after the first cycle of IV Ig. It is possible that positive cross-match in hypersensitised patients was due to antibodies directed against non-HLA antigens expressed in the donor cells. Alternatively antibody activity could be directed against recipient antigens that could be elucidated in an auto cross-match (cross-match between recipient serum and recipient cells), which we did not perform. This patient has lupus, which could explain some cross reactions. The presence of lymphocytotoxic autoantibodies, which can be responsible for elevated PRA and positive cross-match, is not associated with reduced graft survival. Even so, we decided to perform another cycle of IV Ig after which cross-match became negative for AHG-CDC and classic CDC for B and T cells. The high dose administered and the repetition of IV Ig courses in this patient could have contributed to the success obtained. As suggested by others, the dose and repetition of the courses might explain the efficacy of these kind of protocols^{6,10}.

After RT our patient presented persistent donor-specific alloantibody (positive class I and class II antigens alloantibodies) against the serum of the mother. The presence of such alloantibodies after transplantation has been associated with poor outcome after RT¹⁸. The early results of this RT is encouraging; the long term risks of allograft loss and rejection are unknown and may be higher than in cross-match negative patients. Desensitisation protocols offer real hope for patients such as the one in our case study for whom dialysis options were limited and vascular access precarious, reducing life expectancy.

CONCLUSION

The desensitisation protocol used was efficient, allowing RT in a patient with positive cross-match with their potential living donor. Many such patients with an otherwise acceptable living kidney donor never undergo RT due to the presence of preformed antibodies against donor cells resulting in a positive cross-match, and are placed on the deceased donor waiting list, waiting for a suitable donor who never appears.

In hypersensitised patients, such as the patient described above, this could possibly be the sole opportunity for an RT. Kidney transplant in this patient was of vital importance in the face of the exhaustion of vascular access and the impossibility of peritoneal dialysis. This desensitisation protocol seems safe and effective, and could significantly contribute to the expansion of the donor pool for our living donor RT programme.

Conflict of interest statement. None declared.

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