Face Transplantation in a Highly Sensitized Recipient

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ABSTRACT Face transplantation was performed in a highly sensitized recipient with positive preoperative crossmatch and subsequent antibody-mediated rejection. The recipient was a 45-year-old female with extensive conventional reconstructions after chemical burns over the majority of the body. Residual quality of life and facial functions were poor. Levels of circulating anti-human leukocyte antigen (HLA) antibodies were high, and panel reactive antibody score was 98%. A potential donor was identified; however, with positive T and B cell flow crossmatches. The transplant team proceeded with face transplantation from this donor, under tailored immune suppression and with available salvage options. The operation was successful. Plasmapheresis and induction immune suppression (i.e., thymoglobulin followed by mycophenolate mofetil, tacrolimus, and steroids) were provided. Five days later, there was significant facial swelling, rising anti-HLA antibody titers, and unprecedented evidence of C4d deposits on skin. High doses of steroids and thymoglobulin were provided; however, rejection increased such that by day 19 it was diagnosed grade III in the BANFF scale. After stopping thymoglobulin because of serum sickness, combination therapy of plasmapheresis, eculizumab, bortezomib, and alemtuzumab was provided. HLA antibody levels decreased while swelling and redness improved. At 3 months, there were no longer signs of rejection on biopsy.

INTRODUCTION

A positive crossmatch indicates the presence of donor-specific antibodies (DSA), and has been considered a contraindication to solid organ transplantation due to risks of hyperacute rejection and allograft loss.[1,2] Vascularized composite allotransplantation (VCA) dates back 16 years,[3] and up until now has closely followed donor–recipient matching practices established in solid organ transplantation. Thus, all reported hand and/or face allotransplantation cases to date had negative donor/recipient crossmatches.[4,5] By extension, there have been no reports of antibody-mediated rejection (AMR) in VCA,[4,6] in spite of a high incidence of cell-mediated acute rejection.[4] Specifically, there have been no reports of circulating DSA in VCA recipients and no reports of C4d deposition during acute rejection in VCA. C4d is a complement degradation product and marker of AMR that is generated when DSA bind to antigen and activate the complement cascade.[6,7] Owing to this lack of precedent for AMR in VCA, the histopathological scale used to grade the severity of VCA rejection, the 2007 BANFF scale is based on histological features of cell-mediated rejection.[8] Also by extension, all episodes of acute rejection in VCA to date had been treated with high-dose steroids and/or antithymocyte globulins (basiliximab or alemtuzumab), which specifically target cell-mediated rejection.[4,5,9]

Despite lack of evidence for humoral rejection in VCA, clinical practice still mandates an assessment of just how "sensitized" candidates for hand and/or face allotransplantation are. This assessment is depicted by pretransplant panel reactive antibody (PRA) scores.[8] A PRA score is a calculated percentage risk that a given recipient would have a positive crossmatch with a potential organ donor when comparing the human leukocyte antigen (HLA) antibodies found in the candidate's serum with a panel representative of the HLA class I and II molecules found in the general population.[10] Furthermore, standard transplant medicine practice also entails ruling out the presence of DSA against HLA class I and II molecules by both (i) complement-dependent cytotoxicity (CDC) crossmatch and (ii) flow cytometry crossmatch (FCXM).[10]

This is a report on the successful management of the first face transplantation in a recipient with high PRA (i.e., highly sensitized) and positive crossmatch. Evidence of AMR is demonstrated by histological changes and C4d deposition in allograft biopsies with concomitant elevated titers of DSA. Lastly, this report describes the immunosuppression regimen successfully used to abrogate the rejection episode.

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METHODS

The transplant recipient was a 45-year-old female, who 6 years prior had sustained chemical burn injuries to 80% of the body surface area and endured over 50 conventional reconstructive procedures mostly involving split-thickness and full-thickness skin grafting as well as release of contractures. At the time of presentation for face transplant evaluation, the patient demonstrated significant functional impairments, including severe contraction and eversion of the lips, a proximally retracted nose with reduced bulk and volume, lack of functional eyelids, and extensive and painful neck contractures (Fig. 1). All of these contributed to poor quality of life.

This remarkable patient was evaluated and deemed eligible for face allotransplantation by the multidisciplinary VCA team at the Brigham and Women's Hospital (BWH),[11] after a lengthy screening period that involved close institutional review board oversight and active participation of the institution's bioethics team. Some of the strengths this candidate was found to possess were her previous experience as a nurse working on a transplant floor, which made her fully aware of the risks of immunosuppression and her excellent support network. Given her high degree of sensitization, which placed her at increased risk for hyperacute rejection and allograft loss, we implemented a protocol that has been used by our transplant medicine coinvestigators with highly sensitized patients in kidney transplantation with excellent 5-year survival. The patient provided informed consent to enroll in a face transplantation research protocol approved by the Partners Human Research Committee (protocol no. 2008P000550), underwent full screening and was subsequently placed in the transplant wait list. As part of the pretransplant screening, HLA class I and II antibodies in the patient's serum were determined using a flow cytometry-based Luminex 100/200 System (Luminex, Austin, Texas) and single antigen screening beads. The Panel Reactive Antibody (cPRA score was thus calculated.

The New England Organ Bank identified and obtained consent from the next of kin of a brain-dead donor who matched the patient's sex and skin color and texture. Both CDC and FCXM flow cytometric crossmatches between the donor and the recipient were performed. The CDC-crossmatch was performed using donor T and B lymphocytes, mixing recipient serum and donor cells followed by complement. Antihuman globulin was also added to increase sensitivity and the assays were done with serial dilutions of recipient's serum to gain information on the strength of the antibodies detected. The CDC crossmatch was expressed as positive or negative, based on the percentage of dead cells. FCXM was performed by incubating the recipient's serum with donor lymphocytes, fluorochrome-conjugated anti-IgG antibody, and CD3 and CD19 monoclonal antibodies to identify T and B cells. Stained cells were processed by flow cytometry to assess median fluorescence binding of IgG from the recipient's serum.

An immunosuppressive regime was designed based on the crossmatch results that involved traditional induction with antithymocyte globulin (ATG) 1.5 mg/kg, mycophenolate mofetil (MMF) 1g intravenous twice daily, a steroid taper and tacrolimus (Prograf) at 2 mg twice daily (up-titrated quickly to a goal level of 10 ng/mL), and plasmapheresis (therapeutic plasma exchange [TPE]) every other day starting on postoperative day 1 (POD1), with each TPE followed by 10 mg intravenous immunoglobulin (IVIG) to prevent rebound antibody secretion. Postoperatively, the immunosuppression regimen was modified as informed by allograft biopsy results and circulating DSA levels.

Following previously published principles of facial allotransplantation,[12] a robust salvage plan was outlined in the event of loss of the facial allograft. Specifically, efforts were made to preserve the functional units of the recipient's face, such as the functional cartilage of the nose. Extensive lysis of contractures and the use of split-thickness skin grafting over a dermal substitute were deemed highly likely to restore pretransplant appearance and function in the unfortunate event of allograft loss. Potential donor sites for split-thickness skin grafts were identified on the recipient's left arm, forearm, and back.

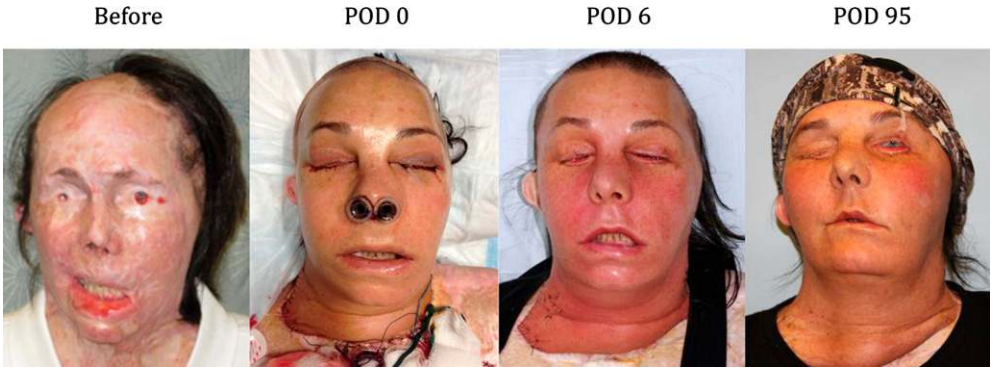

FIGURE 1. Photographs of the recipient before face transplant, and immediately (day 0) and 3 months after surgery. The clinical appearance during acute allograft rejection is also provided, inclusive of biopsy sites on lower right neck (post-operative day 6, POD6). The 3-month time point corresponds to both clinical and histological resolution of the allograft rejection (post-operative day 95, POD95).

Postoperatively, biopsies from the skin of the allograft were obtained every time there were clinical signs of possible allograft rejection. Biopsies were assessed according to the BANFF classification of skin-containing composite tissues.[8] The presence or absence of C4d in the allograft was determined by both immunoperoxidase and direct immunofluorescence staining of biopsy samples collected in formalin and Zeus transport solution, respectively. All biopsy specimens were received, processed, and tested by the Department of Pathology at the BWH; in particular, the immunofluorescence microscopy was developed and performance characteristics were determined by the Immunohistochemistry Laboratories in the Department of Pathology at the BWH.

Serum samples were acquired concomitant to allograft skin biopsies and assessed for DSA levels using flow cytometry-based Luminex 100/200 System and single antigen screening beads as described above.

RESULTS

Luminex solid phase prescreening of the stored recipient's serum while on the wait list revealed antibodies to a significant number of HLA class I and II antigens, and a cPRA of 98. The patient waited in the list for 14 months, which at that time was 3 to 4 times longer than any of the prior VCA recipients at BWH. After the contingency plans outlined above were put in place, the transplant team decided to transplant the face of a donor against whom the recipient may have had DSA (i.e., positive crossmatch). A donor was identified shortly later and was accepted based on matching blood group, age, sex, and skin color.

A sample of recipient's serum taken on the same day of—but before—the transplant operation confirmed the presence of DSA, specifically, anti-HLA A2, A32, B57, BW4, DQ7, DQ9, and DR4. Anti-HLA DQ7, DQ9, and DR7 were present in the undiluted serum only, but anti-HLA A32, B57, and BW4 were still present at 1:32 dilution. The T cell flow crossmatch was positive at DFU 1,428 (cutoff of positivity is >60) and the B cell crossmatch was 1,850 (cut off > 1,000). Initial T cell CDC tests conducted in serum taken from the recipient 3 months prior and stored since yielded negative results, whereas when conducted using sera drawn on the day of transplantation the results were weakly positive with a cell death score of 20%.

The operation was performed uneventfully (Fig. 1). Peri- and postoperative immunosuppressive management was informed by DSA levels and allograft biopsy results. The standard induction regimen (centered on abrogating cell-mediated rejection) was bolstered with TPE and IVIG every other day, starting POD1, in an effort to reduce DSA burden and associated risks of AMR.

The allograft underwent one lengthy and complex rejection episode in the immediate postoperative period. This episode started by POD5, when the patient presented with significant lower facial swelling and erythema (Fig. 1). Induction therapy and three rounds of PTE had been completed. Biopsies of the allograft skin showed no evidence of cellular rejection. However, circulating DSA were stronger when compared with the pretransplant results. Anti-HLA A2, A32, B57, and DR7 remained present at 1:32 dilution (Fig. 2). Out of concern for humoral rejection, TPE was stopped after the fourth run and switched to complement blockade with eculizumab once per week, as well as administered a second steroid pulse and taper. MMF and tacrolimus remained unchanged.

By POD12, biopsies from the allograft skin showed perifollicular lymphocytic infiltration consistent with BANFF grade I rejection. As the patient was already undergoing a second steroid pulse, the team made no alterations in management. By POD15, however, redness and swelling were unchanged and there was more pronounced lymphocytic infiltration with exocytosis into epithelium, consistent with BANFF grade II rejection. In addition, for the first time signs of possible AMR became evident, as suggested by intraluminal neutrophils on the specimens of allograft skin and further supported by findings on immunofluorescence microscopy of capillaries in the papillary dermis and around the eccrine glands. Small arteries and arterioles were also reactive for C4d (3 to 4+/4+). Of note, the tissue was negative for C3 and C1q deposits and there was no evidence for immune complex deposition (Figs. 3 and 4). By POD19, allograft skin biopsies were graded as BANFF grade III rejection (Fig. 5). There were foci of epidermal lymphoid exocytosis and early primarily follicular apoptosis that were slightly more prominent. Again, occasional intraluminal neutrophils without frank necrotizing leukocytoclastic vasculitis were noted and the strength and distribution of C4d

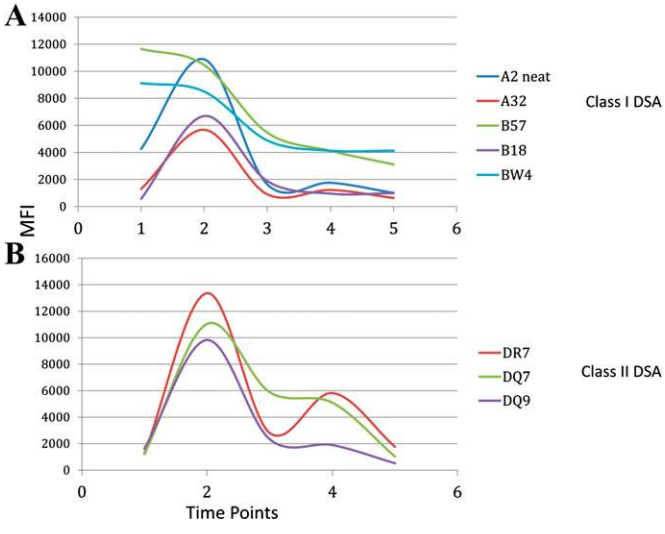

FIGURE 2. Histograms showing donor-specific anti-HLA (A) class I and (B) II antibody levels in the recipient's serum as determined by Luminex solid-phase single-antigen assays. The DSA levels are those measured from the neat serum samples drawn in the postoperative period. Time on the x-axis is not to scale. Time points 1 to 5 correspond with post-operative days 0, 20, 32, 39, and 47, respectively.

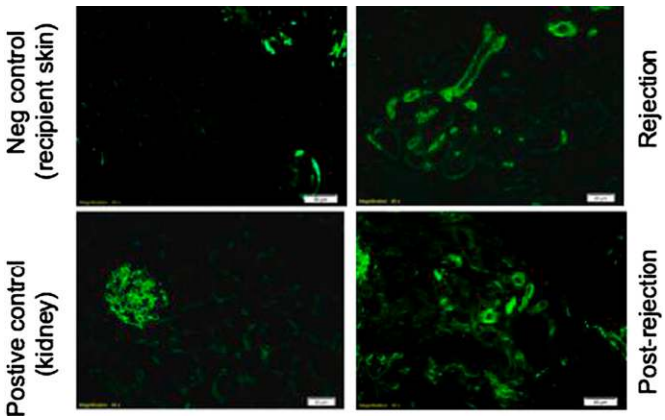

FIGURE 3. C4d deposition in skin microvasculature by direct immunofluorescence. Direct immunofluorescence highlights C4d deposition in superficial and deep dermal microvasculature before and during rejection (top right) and with residual positivity only in deeper vessels in postrejection phase (bottom right). Uninvolved recipient skin (top left) and active glomerulonephritis kidney (bottom left) were performed as negative and positive controls, respectively.

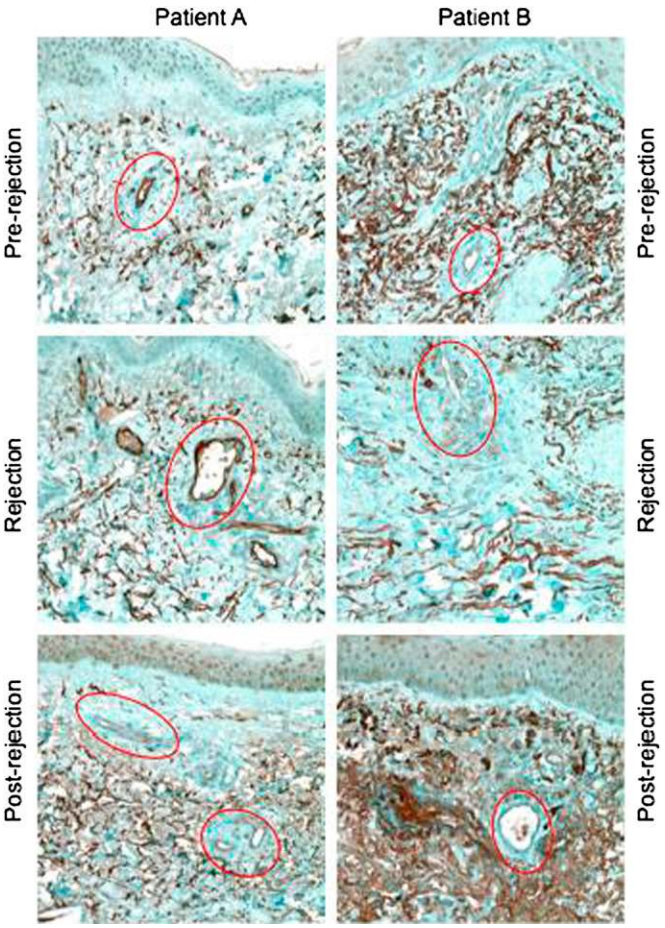

FIGURE 4. C4d deposition in skin microvasculature by immunohistochemical stains. Immunohistochemical stains highlight C4d deposition in superficial and deep dermal microvasculature (circled) before (top) and during rejection (middle) in our presensitized face allotransplant recipient with elevated DSA (labeled patient A; left column), C4d was largely negative in the patient's treated, postrejected specimen (bottom). The right column shows biopsy specimen from a prior unsensitized face allotransplant recipient with no detectable DSA (labeled patient B; right column); no C4d deposition in microvasculature was detected in either the pre-, during, or postrejection allograft biopsies of patient B. Nonspecific background of elastic fibers staining present in all specimens.

staining remained just as prominent as that seen on POD15. There was also a further increase in circulating DSA levels: anti-HLA A2, A32, B18, B57, Bw4, DR7, DQ7, and DQ9 were all detected in the neat and 1:8 serum samples, and anti-HLA A2, B57, DR7, DQ7, and DQ9 in the 1:32 dilution samples. At this point, the immunosuppression regimen was modified. Specifically, the following interventions were implemented in an effort to counteract the humoral component of the acute rejection: (i) 6 additional runs of TPE and IVIG over the course of 8 days, (ii) eculizumab administration following TPE on POD20, 22, and 27, and (iii) bortezomib administration following TPE on POD22 and 25. Other interventions were carried out to address the cell-mediated component of the acute rejection, namely: (i) a third steroid pulse and taper over the course of POD16 to 25, (ii) another 6.5 mg/kg of ATG over POD19 to 24, and (iii) extracorporeal photopheresis daily on POD27 to 29. Of note, ATG was held on POD25 because of signs of serum sickness in the patient's knees and ankles. The immunosuppression regimen was bolstered with a one-time dose of 15 mg of alemtuzumab on POD29 (Fig. 5).

These adjustments in immunosuppression propitiated a slow reduction in erythema of the allograft. By POD29, there was significant decrease in circulating DSA levels. Although anti-HLA A2, A32, B18, B57, Bw4, DQ7, and DQ9 were found in the neat serum samples, only anti-HLA B57 and DQ7 were detectable at 1:8 dilution and no DSAs were detectable at 1:32 dilution. Allograft biopsies were downgraded to BANFF grade II rejection and showed less C4d immunoreactivity (Fig. 5). The above described immunosuppressive regime was continued for 2 further weeks: a fourth round of TPE and IVIG was performed on POD33 to 36, eculizumab was administered weekly, and 2.2 mg of Bortezomib were administered every 4 days. Maintenance with tacrolimus (target levels of 8–12 ng/mL), MMF, and steroids continued as well (Fig. 5).

By POD39, there were no DSA detected in the 1:32 or 1:8 sera dilutions, and allograft skin biopsies were unchanged in terms of BANFF grade and C4d deposition. The recipient was discharged from the hospital on POD41 on a maintenance regime of tacrolimus, MMF, and low-dose steroids, as well as TPE and IVIG twice per week. On POD51, allograft biopsies showed no evidence of overt vasculopathy, and only superficial/mid-dermal perivascular lymphocytic infiltrate suggestive of BANFF grade I. C4d staining persisted at that time, but the only detectable DSA were anti-HLA A2, B57, Bw4, DR7, and DQ7 found in the neat serum samples (Fig. 2). Although the team stopped TPE, circulating DSA levels continued their decline (anti-HLA B57,

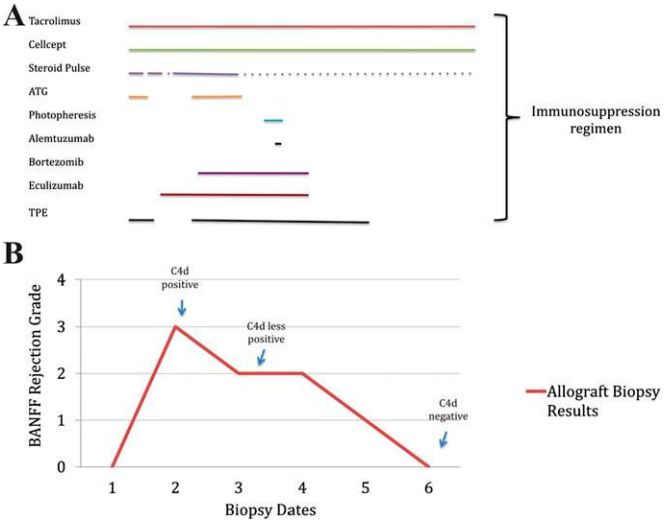

FIGURE 5. Graph showing (A) the immunosuppression regimen over time as it related to allograft biopsy results and (B) the BANFF histological grades of rejection and C4d immunoreactivity as determined on allograft biopsies in the postoperative period. Time on the x-axis is not to scale; biopsy dates 1 to 6 are post-operative days 0, 19, 25, 39, 53, and 116, respectively.

DR7, and DQ7 being those detectable on POD68) and the patient continued to exhibit clinical improvements with near complete resolution of erythema, swelling, and lymphadenopathy (Fig. 1D). Allograft biopsies obtained on POD89 showed only sparse superficial perivascular lymphocytic infiltrate, positive C4d staining only on the vessels around the eccrine glands of the dermis, with the papillary dermis being largely negative for C4d immunoreactivity. By POD116, there was no longer evidence of active cellular or AMR. At this point, this rejection episode was deemed resolved. Routine allograft biopsies taken subsequently yielded normal (i.e., rejection) results, until a biopsy taken on day 358 demonstrated perivascular chronic inflammation with minute focus of epidermal spongiosis associated with vacuolar interface change and exocytosis, consistent with BANFF grade II allograft rejection. The patient was admitted to the hospital for steroid bolus treatment (solumedrol 500 mg intravenous every day ×3); however, 1 month later, the findings of BANF II rejection persisted. Topical steroids were added. Clinical and histopathological presentation improved slowly and rejection was considered resolved by the beginning of the 14th postoperative month. On the 21st month, an allograft biopsy showed grade II rejection, which was treated with oral steroids, 100 mg for 5 days. Finally, on the 24th postoperative month there was another acute rejection episode, graded Banff II/III which resolved after 1 week of topical treatment with clobetasol ointment.

DISCUSSION

With regards to VCA, this case yielded the first observations of: (i) transplantation in a highly sensitized recipient with positive donor–recipient crossmatch, (ii) evidence of AMR, and (iii) successful management of AMR.

AMR is therefore both possible and relevant in VCA, a point that has been refuted based on the absence of DSA and/or C4d deposition in prior VCAs.[4,6,13] It is important to note, however, that all prior VCAs were performed in patients with negative donor–recipient crossmatches.

The hereby described findings call for a revision of the current BANFF working classification for VCA.[8] When the Banff classification was drafted in 2007, there was consensus that "several pieces of histologic and clinical information" needed to be gathered to define AMR in VCA,[8] including "the presence of C4d deposition and its relationship with donor-HLA-specific antibodies, the presence of vasculitis, neutrophilic margination, thrombi and necrosis, a complete history of sensitization (e.g., PRA, crossmatch results, transfusions, pregnancies, and previous allografts), and the presence or absence of autoantibodies and T- and B-cell crossmatch performed before transplantation."[8] All of these pieces of information have been outlined in this case report, and as such they may help the eventual revision of the BANFF classification so as to define and incorporate AMR in the context of VCA rejection.

Sensitization is a common scenario in burn patients and in those with a history of allograft failure, and up until now had been considered a contraindication to VCA. This report may provide a step toward revisiting and expanding the criteria of eligibility for face allotransplantation. Face transplantation in a sensitized patient with a positive crossmatch was possible and could be managed by careful adjustments of immunosuppression with drugs currently used in solid organ transplantation; however, the rescue drug protocol was complex and costly, and needs further refinement. Although the patient continues to do well clinically with no evidence of rejection on biopsies 6 months postoperative, time will better reveal the eventual prognosis and help inform management and course.

CONCLUSION

Face transplantation was performed in a highly sensitized recipient with positive donor–recipient crossmatch. The 2-year postoperative outcomes suggest that face transplantation in this patient population is possible and manageable. The incidence of episodes of acute rejection in this patient has been comparable to those reported in the literature for other cases of vascularized composite allotransplantation, and have been managed successfully. There is no evidence of chronic rejection. However, further refinements to the immunosuppression protocol and longer followup are needed.

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