

Two Case Reports of Successful withdrawal of mycophenolate mofetil after living donor  
lobar lung transplantation

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## **INTRODUCTION**

In most cases of lung transplantation, immunosuppression is maintained using calcineurin inhibitors (CNIs), anti-metabolites and steroids. Lung transplant recipients on such a regimen of triple immunosuppressants are susceptible to infectious diseases. Moreover, many prophylactic antibiotics and laboratory studies are needed, including monitoring of immunosuppressant levels and respiratory function tests, to prevent or predict allograft rejection. However, serum immunosuppressant levels alone may not accurately reflect immune status. ImmuKnow® (Cylex, Columbia, MD) is a novel and promising in vitro assay for measuring the cell function of stimulated T cells.<sup>1</sup> Bhorade et al.<sup>2</sup> reported that ImmuKnow® levels were lower in lung transplant recipients with infection than in non-infected recipients. We report two clinical cases in which mycophenolate mofetil (MMF) was successfully withdrawn after living donor lobar lung transplantation while monitoring patient immune function with the ImmuKnow® assay.

## **CASE REPORT**

### **Case 1**

A 43-year-old woman underwent living donor lobar lung transplantation (LDLLT) for pulmonary alveolar proteinosis. The left lower lobe was donated by her older brother, while the right lower lobe was donated by her husband. Both donors appeared healthy. Postoperative course was uneventful, and she was discharged without any oxygen support. She had been receiving a standard triple immunosuppressant regimen, comprising tacrolimus (target trough level, 15-20 ng/ml for the first 3 months, followed by 10-15 ng/ml), MMF (1500 mg/day), and prednisolone (0.4 mg/kg/day for the first 6 months, followed by 0.2 mg/kg/day). Our prophylactic strategies for fungal, viral, and protozoan infections were oral itraconazole at 100 mg/day, valganciclovir at 900 mg/day, and trimethoprim-sulfamethoxazole at 1 g every other day, respectively. Six months

after transplantation, the patient developed invasive pulmonary aspergillosis (IPA). MMF was withdrawn immediately, and the trough level of tacrolimus was reduced to around 5-8 ng/ml to allow the immune status of the patient to recover and battle this fatal infection. The patient was treated with oral voriconazole at 300 mg twice daily, inhalation of amphotericin B at 10 mg 5 times daily, and intravenous micafungin at 300 mg/day. Fortunately, her condition improved over 3 months of hospitalization, and she was discharged without any symptoms. Oral voriconazole and inhaled amphotericin B were continued as prophylaxis for 9 months. Details of the clinical course have been reported previously.<sup>3</sup> Immune function in this patient was monitored using the ImmuKnow® assay from 14 to 37 months after transplantation (Fig. 1). Local ethics committee approval for this clinical research was obtained prior to commencement of this study. During this period, T-cell immune function largely remained within the moderate range. After recovery from IPA, immune function remained within the moderate range despite withdrawal of MMF. No rejection or other infection was seen during this period. We have retained this strategy to avoid recurrence of IPA. No rejection was identified from respiratory function testing and no other infections have been encountered after achieving moderate immune function (Fig. 2).

## **Case 2**

A 24-year-old man underwent LDLLT for cystic fibrosis. He had paranasal sinusitis, but curative surgery had already been performed before transplantation. The left lower lobe was donated by his father, while the right lower lobe was donated by his uncle. After LDLLT, primary graft dysfunction occurred in the left lung due to the some influence of mild alcoholic liver cirrhosis. As a result, immediate single lobar re-transplantation was performed using the left lower lobe donated by his sister.<sup>4</sup> Postoperative course after the redo operation was uneventful, and the patient was discharged without any oxygen

support. He had been receiving treatment under the same strategy seen in Case 1, with a standard triple immunosuppressive and prophylactic regimen for infectious disease. From 5 months after transplantation, he experienced several episodes of *Pseudomonas aeruginosa* pneumonia derived from the paranasal sinus sinuses (as suggested by findings from Gram-staining) that had been treated before transplantation. Infection was successfully treated with administration of inhaled tobramycin.

Immune function in the early postoperative period showed low-level immunosuppression, so the decision was made to withdraw MMF to prevent infection and allow redo-surgery for sinusitis. Immune function gradually increased to moderate levels after withdrawal of MMF and sinusitis surgery (Fig. 3). Local ethics committee approval for this clinical research was also obtained prior to lung transplantation. No rejection was evident on respiratory function testing and no other infections were seen after achieving moderate immune function (Fig. 4).

## **DISCUSSION**

This report describes two clinical cases in which MMF was successfully withdrawn after LDLLT while monitoring the immune function of patients using the ImmuKnow® assay. Our report shows that use of just two immunosuppressants, comprising a CNI and a steroid, could maintain moderate immune function after LDLLT.

Optimization of the dosages of immunosuppressants is always a key issue. LDLLT patients show a lifelong risk of allograft rejection, possibly because of fluctuations in immune status with low-level immunosuppression. On the other hand, patients are susceptible to infection because of frequent exposure to infectious microorganisms (such as bacterial, viral, and fungal) under conditions of high-level immunosuppression, despite administration of prophylactic agents. In addition, triple immunosuppressant regimens result in a number of morbidities, including renal dysfunction, diabetes, and

digestive disorders. According to the registry of the International Society for Heart and Lung transplantation,<sup>5</sup> approximately 95% of recipients are still receiving a triple immunosuppressive regimen as of 1 year after lung transplantation. Some case reports have described successful withdrawal of steroids in lung transplant recipients during a short period follow-up.<sup>6,7</sup> However, to date, no clinical evidence has been accumulated to support maintaining lung transplant patients on two immunosuppressant regimens.

ImmuKnow® is a novel and promising in vitro assay for measuring the cell function of stimulated CD4<sup>+</sup> T cells, and has been licensed by the Food and Drug Administration for monitoring the immune systems of transplant patients.<sup>1</sup> This assay functions on the principle of incubating target T cells with phytohemagglutinin and measuring the production of intracellular adenosine triphosphate (ATP) as a reflection of cell metabolism.<sup>8</sup> Immune status is expressed as strong ( over 525 ATP ng/ml), moderate (524-226 ATP ng/ml), or low immune function (under 225 ATP ng/ml ). Recently, some clinical reports have described promising results using this assay in lung transplantation patients.<sup>2,9</sup> Such reports have indicate to us that quantification of immune function could help determine patients at risk of infection or allograft rejection.

In the present two cases, each patient had received the standard triple immunosuppressant therapy (CNI, MMF, and steroid) after LDLLT. We had to reduce the level of immune suppression because both patients suffered from intractable infectious disease. On the other hand, we became very concerned that the patients might develop allograft rejection with discontinuation of MMF, which was considered a key immunosuppressant during this treatment. We therefore started to measure immune function using the ImmuKnow® assay as an objective tool monitoring 'real' immune status. This assay showed that immune function in both cases had largely been maintained within the moderate range, which encouraged us to continue withholding MMF.

Based on these results, the immune function of patients remained within or close to the moderate range despite use of only two immunosuppressants (a CNI and prednisolone) after recovery from infectious disease. When monitoring of immune status is feasible, an immunosuppressant regimen without MMF might show a reduced risk of side effects that can sometimes result in severe complications (such as gastrointestinal disorder and myelosuppression).

Several limitations must be considered when interpreting the present findings. Follow-up in these case reports was short and immune function is very complicated, so evaluation of CD4<sup>+</sup> T-cell function alone is not necessarily representative of total immune function in each patient. Another problem is that, in general, most LDLLTs are undertaken between relatives and extensive immunological matching may take place beforehand to select the most appropriate donor. Such practices might have contributed to the ability to maintain moderate immune function without MMF in the present cases.

In conclusion, immune evaluation using ImmuKnow® could be useful for monitoring immune status in patients. Although this report describes only two cases and follow-up was short, this method showed that moderate immune status can be maintained using only two immunosuppressants in some patients after LDLLT, reducing susceptibility to infectious disease. Further study is needed to determine the scope of the role ImmuKnow® can play in optimizing the immune status of lung transplant patients.

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## **Disclosures and Freedom of Investigation**

We have no personal conflicts of interest to declare and no outside support was received for this research.

## REFERENCES

- [1] Kowalski RJ, Post DR, Schneider MC, et al. Immune cell function testing: an adjunct to therapeutic drug monitoring in transplant patient management. *Clin Transplant* 2003;17:77-88.
- [2] Bhorade SM, Janata K, Vigneswaran WT, et al. Cylex ImmuKnow assay levels are lower in lung transplant recipients with infection. *J Heart Lung Transplant* 2008;27:990-4.
- [3] Tagawa T, Yamasaki N, Tsuchiya T, et al. Living-donor lobar lung transplantation for pulmonary alveolar proteinosis in an adult; report of a case. *Surg Today* 2011;41:1142-4
- [4] Tagawa T, Yamasaki N, Tsuchiya T, et al. Immediate single lobar retransplantation for primary graft dysfunction after living-donor lobar lung transplantation: report of a case. *Surg Today* 2011;41:1447-9
- [5] Christie JD, Edwards LB, Kucheryavaya AY, et al. The registry of the International Society for Heart and Lung Transplantation: Twenty-eighth Adult Lung and Heart-Lung Transplant Report-2011. *J Heart Lung Transplant* 2011;30:1123-32.
- [6] Borro JM, Solé A, Torre DL, et al. Steroid withdrawal in lung transplant recipients. *Transplant Proc* 2005;37:3991-3.
- [7] Shitrit D, Bendayan D, Sulkers J, et al. Successful steroid withdrawal in lung transplant recipients: result of pilot study. *Respir Med* 2005;99:596-601.
- [8] Kowalski RJ, Post DR, Mannon RB, et al. Assessing relative risks of infection and rejection: a meta-analysis using an immune function assay. *Transplantation* 2006;82:663-8.
- [9] Husain S, Raza K, Pilewski JM, et al. Experience with immune monitoring in lung transplant recipients: correlation of low immune function with infection. *Transplantation* 2009;87:1852-7.



## FIGURE LEGENDS

**Figure 1.** The gray bar shows immune function from 14 to 37 months after transplantation. During this period, T-cell immune function largely remained within the moderate range after recovery from invasive pulmonary aspergillosis. The line plot shows tacrolimus trough levels, which were controlled to low levels (around 5-8 ng/ml). Horizontal lines represent cut-off levels for strong (ATP, over 525 ng/ml), moderate (ATP, 524-226 ng/ml), and low immune function (ATP, under 225 ng/ml). IPA, invasive pulmonary aspergillosis. ATP, adenosine triphosphate

**Figure 2.** The gray bar shows immune function from 4 to 28 months after transplantation. After withdrawal of MMF and a redo sinusitis operation, immune function remained above the threshold for moderate immune function during this period. Horizontal lines represent cut-off levels for strong (ATP, over 525 ng/ml), moderate (ATP, 524-226 ng/ml), and low immune function (ATP, under 225 ng/ml). MMF, mycophenolate mofetil. ATP, adenosine triphosphate

**Figure 3.** Case 1. Forced expiratory volume in 1 s after lung transplantation. No significant deterioration of respiratory function was seen during this period.

**Figure 4.** Case 2. Forced expiratory volume in 1 s after lung transplantation. No significant deterioration of respiratory function was seen during this period.

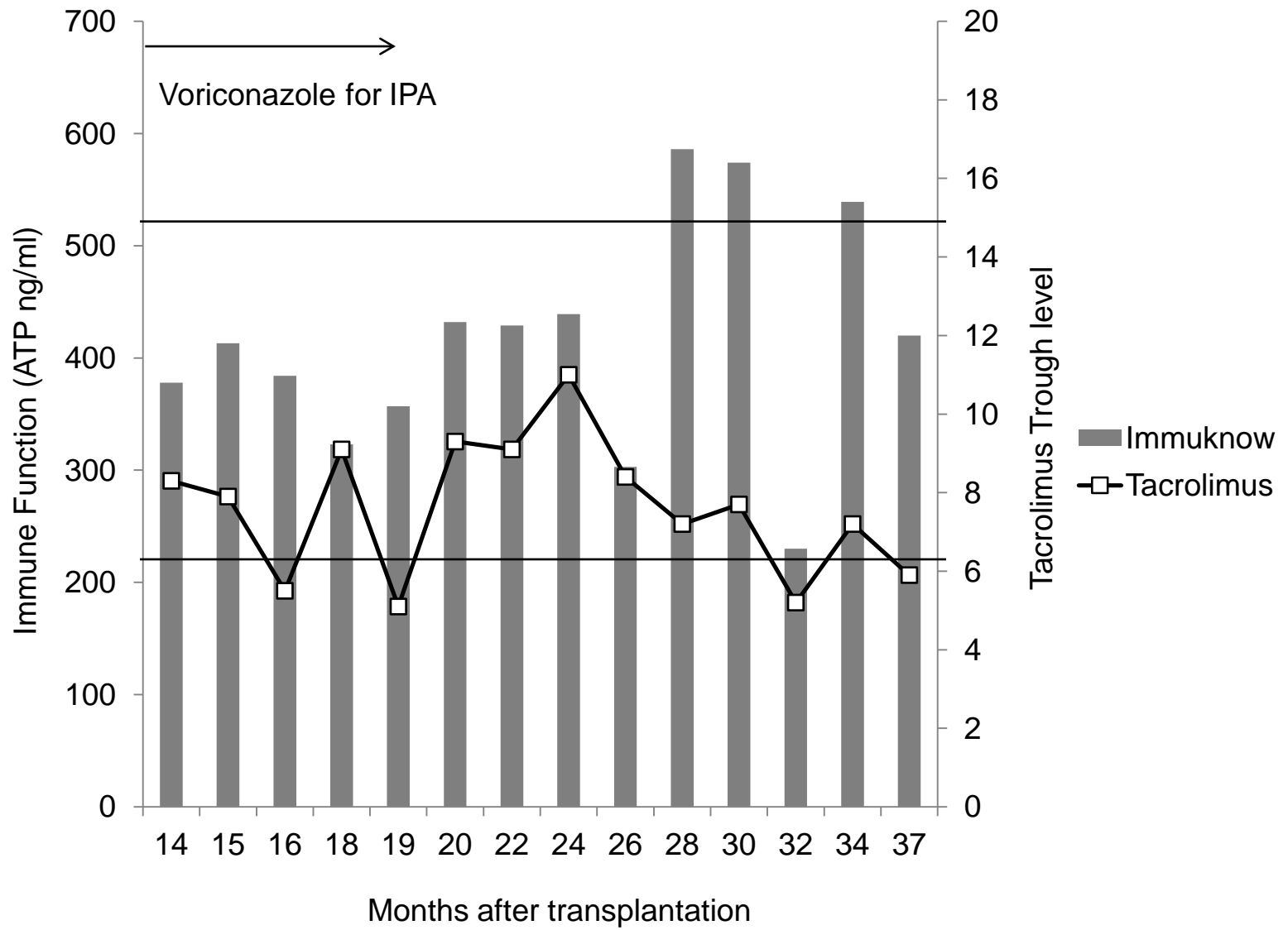


Figure 1

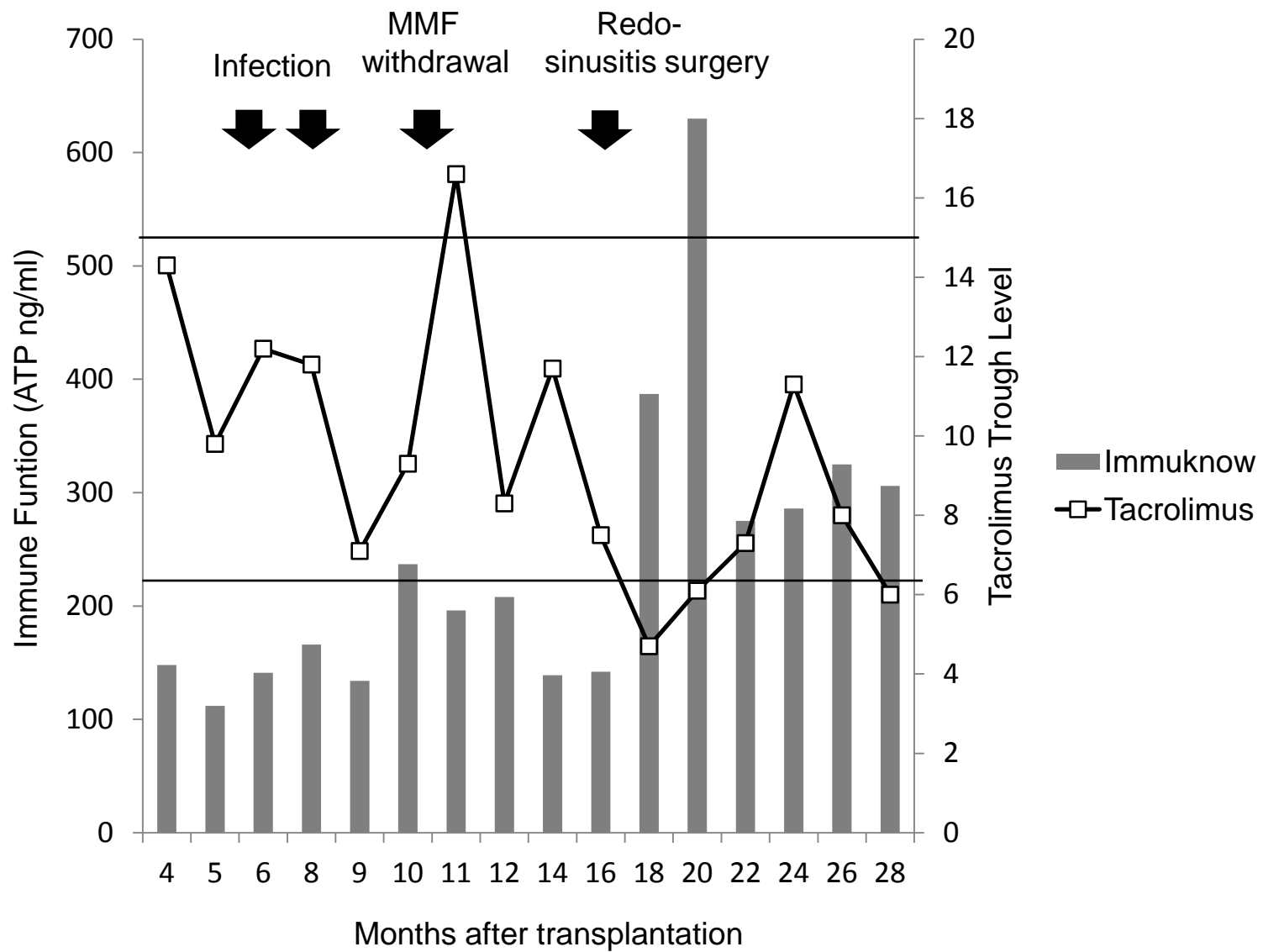


Figure 2

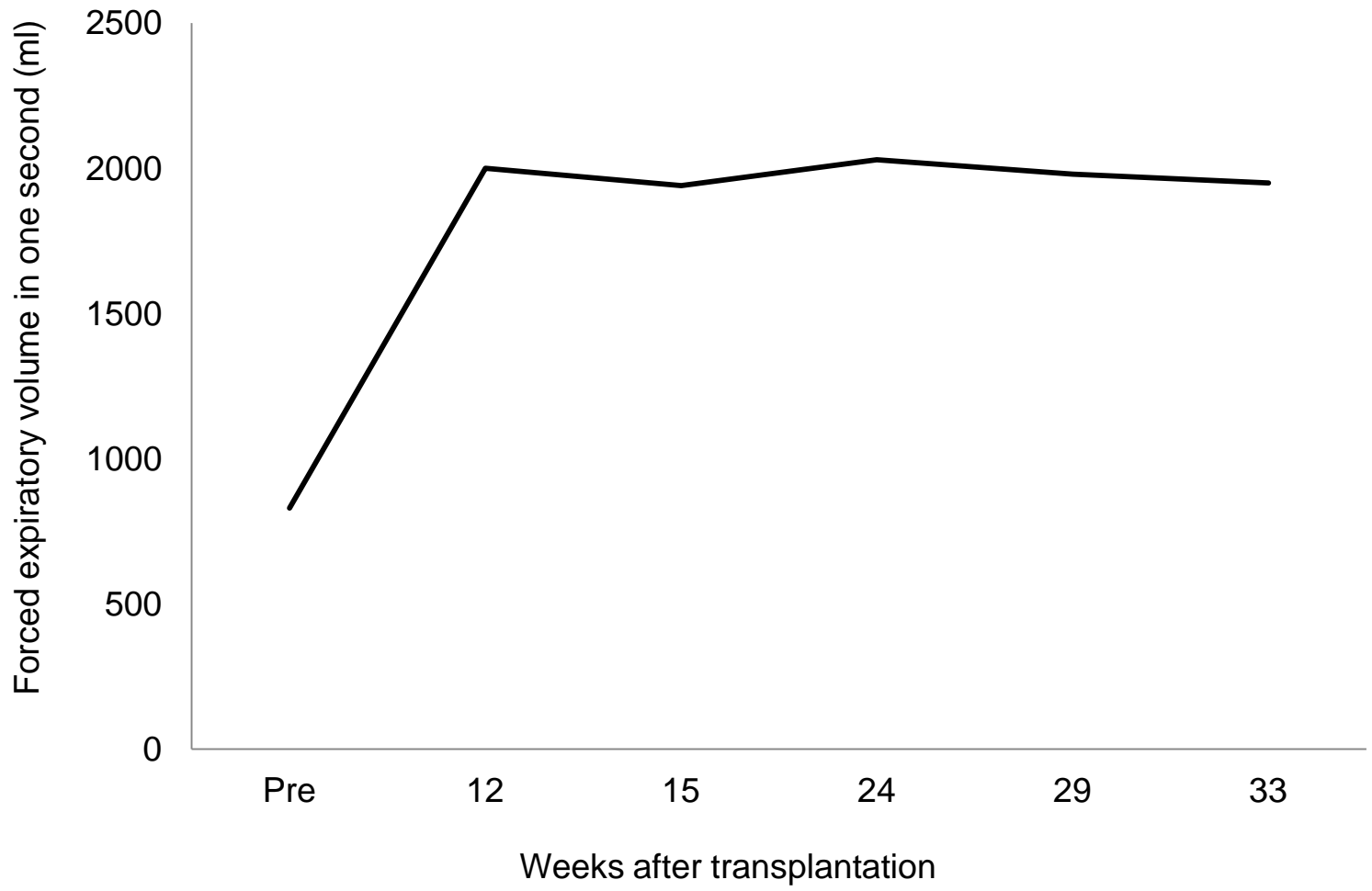


Figure 3

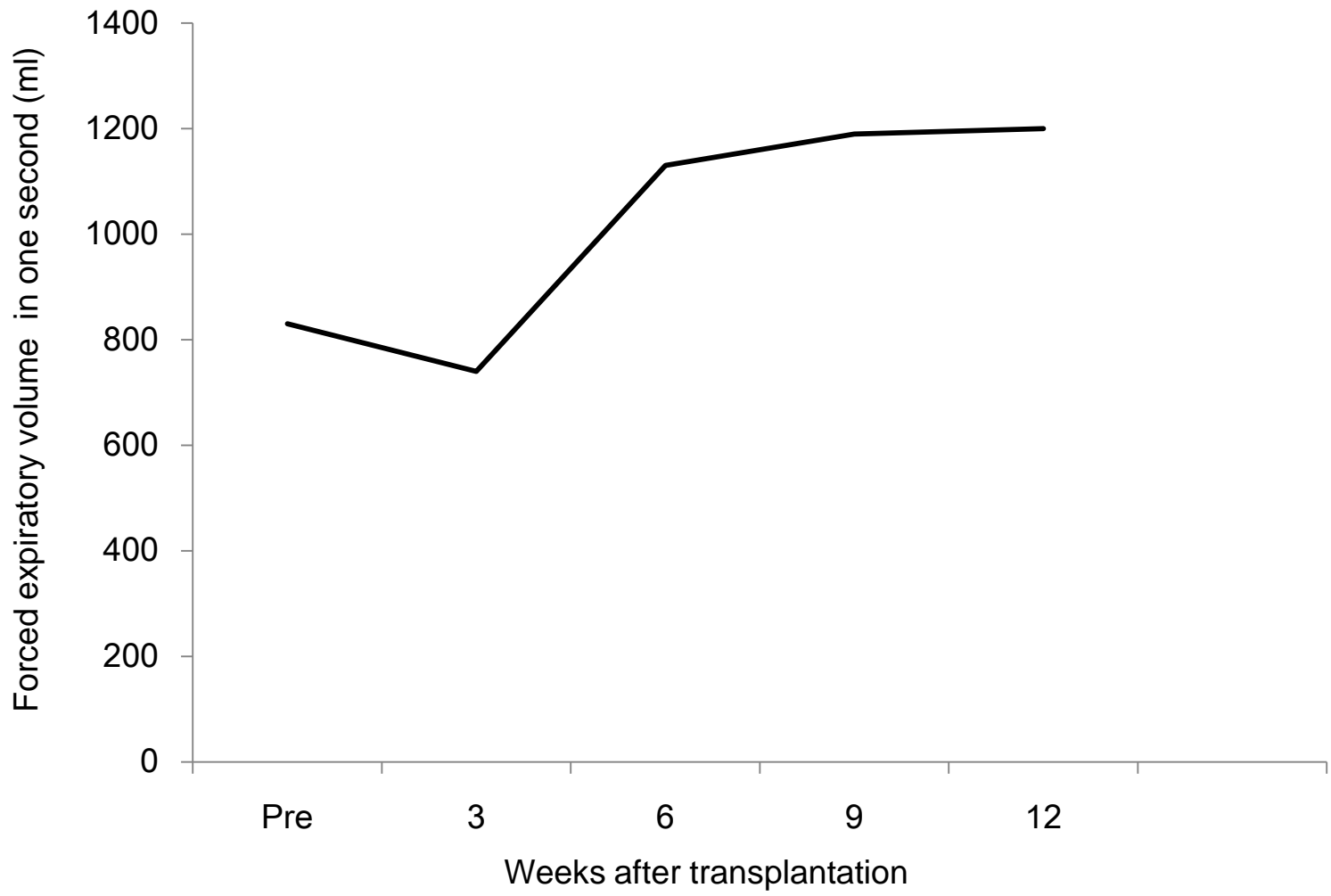


Figure 4