Peer Review File
Article information: https://dx.doi.org/10.21037/jtd-22-1760

Reviewer A

Sohn et al described their experience with intravenous tacrolimus after lung transplantation. The authors are aware of the major limitations of their study and they stated clearly them on page 8, lines 19 - 24.
I have the following additional remarks:
: The authors appreciate the reviewer's encouraging comments. Regarding to your comments, we revised our manuscript based on your comments and suggestions.

Comment 1. The authors did not report what happened when Tacrolimus was switched to oral administration. Did the trough levels worsen? The authors reported the Tacrolimus levels for the first 14 days after transplantation: However, the median hospital stay time amounted to 45 days (Table 1).
Reply 1. We thank the reviewer for valuable comments. As described in the Method section, after reaching a steady-state, tacrolimus dosing was switched to an oral equivalent daily dose in patients assured of adequate oral intake. As described in the result section, steady status was reached and converted to oral form within a median of 6 (5-8) days, and through level was well maintained as shown in Figure 1B. we added this information in the results section.

Comment 2. The authors should report the incidence of acute cellular rejection also after hospital discharge. Were transbronchial biopsies performed?
Reply 2. In our institute, the surveillance bronchoscopies with transbronchial lung biopsies are not routinely performed, but when indicated by symptoms and sign. Fortunately, however, there was no acute cellular rejection in our patietns during the study period. The reason why we only report the frequency of acute cellular rejection within one month of transplantation was to determine whether it was related to the through level at the initial 1-2 weeks after transplantation and the occurrence of the early rejection. However, it is difficult to think that it is related to the occurrence of late rejection during long-term follow-up and the through levels during the first 2 weeks after lung transplantation.

Comment 3. Similarly, are data on the incidence of chronic lung allograft dysfunction (CLAD) available?
Reply 3. Yes, the incidence of CLAD is available, but not all transplant recipient could be evaluated for CLAD. According to the ISHLT report in 2019, there are patients who are not included in the definition of CLAD. Excluding these patients (n = 29), 8 of 38 (21%) patients were diagnosed with CLAD during the follow-up period. However, this information was also not added to the results because there was little association between the levels of tacrolimus within the first two weeks of lung transplantation and the various causes of CLAD.

Comment 4. Adverse events: how was the neurotoxicity evaluated? Were the cases of acute kidney injury due to tacrolimus overdosage or to other causes (e.g. haemodynamic instability, effect of other nephrotoxic drugs...).
Reply 4. We apologize for the lack of clarity. But, we could not evaluated the tacrolimus related nephrotoxicity itself. As you mentioned, it is difficult to distinguish between tacrolimus-related nephrotoxicity and the various acute kidney injuries that occur after transplantation, so we are simply reporting the frequency of acute kidney injury following tacrolimus infusion.

Comment 5. Overall, it is not really clear the aim of this study. It is known that the intravenous use of tacrolimus is associated with a better stability of the drug through levels. However, in this study, the effects of this stability on the outcomes did not really emerge/were not investigated.
Reply 5. We apologize for the lack of clarity. As described in the background section, there are no clear guidelines on how long duration needed to achieve the therapeutic range with continuous intravenous administration in the early phase of lung transplantation. Compared to other studies in the literature, therefore, we put our interest not in the clinical outcome but more in the achievement of therapeutic range using continuous intravenous administration and the clinical pharmacist's daily intervention. We modified the objective of this study in the revised manuscript.

Comment 6. Page 3, line 16: did the authors mean "acute cellular rejection"?
R6. We apologize for our carelessness. It shoud have been 'acute cellular rejection'. We corrected the typo in the revised manuscript.

Comment 7. Page 6, lines 7 and 8: the sentence "...one died after one tacrolimus though concentration was excluded from the analysis" is not really clear to me.
Reply 7. This means that one patient died early and was excluded from the analysis because the patient had only one measure of tacrolimus trough concentration. We modified the sentence.

Reviewer B

The manuscript is well written. While there is documented information regarding oral/sublingual tacrolimus after lung transplant, there is little published regarding intravenous tacrolimus. This manuscript may serve as a guide to elaborate on the timeline to achieve a therapeutic trough of tacrolimus if the intravenous form is used. This time to therapeutic range of the patients in the study receiving intravenous tacrolimus is similar to the expectation for oral/sublingual tacrolimus and therefore this seems like a reasonable approach/alternative. However, providers are limited in achieving a quick time to therapeutic range in new transplant recipients due to concerns with adverse effects, particularly nephrotoxicity from rapid acceleration of tacrolimus levels.
I have a few suggestions for the author to elaborate on to strengthen the manuscript
: The authors really appreciate the reviewer's encouraging comments. Regarding to your comments, we revised our manuscript based on your comments and suggestions.

Comment 1. The level of acute kidney injury (31% or roughly 1/3 of recipients) seems high and this risk of renal dysfunction is a reason that other centers are often reluctant to utilize intravenous tacrolimus. It would help to elaborate if this acute kidney injury was merely transient (an increase in serum creatinine that resolved within 1-2 days) or rather more persistent (minor loss that resulted in prolonged kidney injury or kidney dysfunction).
Reply 1. We apologize for the lack of clarity. As described in the results section, most of acute kidney injury was transient and recovered within days, but 10 (13%) patients with acute renal failure needed RRT. However, all of these patients were finally liberated from the RRT before discharge from ICU.

Comment 2. You mention a lack of "severe adverse events" in the Discussion Line 9 yet did

not evaluate the incidence of infections. Furthermore, it would seem reasonable to address serious infections that may have occurred in the patients since it is also mentioned that this is often a concern with early initiation of high doses of tacrolimus (Discussion Line 18).
Reply 2. In this study, tacrolimus administration commenced at a low dose of 0.01 mg/kg/day, which is the lowest dose of the continuous infusion suggested. Therefore, the risk of infection was not expected to increase, and there was no serious infection causing sepsis and shock. We added this information in the revised manuscript.

Comment 3. Finally, the introduction (Lines 12-13) mentions that intravenous route may be preferable in patients ventilated on with gastroparesis. However, it is well documented that these sublingual/ feeding tube routes may be useful in these specific situations and the reference cited also mentions this. Please rephrase this sentence to describe advantages to using intravenous while acknowledging that patients can use sublingual tacrolimus while intubated or in cases of severe gastric emptying.
Reply 3. We thank the reviewer for valuable comments. We modified the sentence as your suggestion.

Reviewer C

The issue raised by the authors is undoubtedly interesting. However, the methods used are not yet the best.
The following points need to be taken into consideration and be reviewed:
: The authors really appreciate the reviewer's valuable comments. Regarding to your comments, we revised our manuscript based on your comments and suggestions.

Comment 1. Title: The title should better reflect the type of study conducted.
Reply 1. We apologize for the lack of clarity. We modified our title as 'Clinical pharmacokinetic study of tacrolimus in continuous intravenous administration for lung transplantation'.

Comment 2. Line 16: There must be an error in this sentence. It seems the word "rejection" was forgotten.
Reply 2. We apologize for our carelessness. It shoud have been 'acute cellular rejection'. We corrected the typo in the revised manuscript.

Comment 3. Line 3: Reference [1] is somewhat outdated. It would be advisable to try to find a better and more recent one for accompanying the text.
Reply 3. We thank the reviewer for valuable comments. We updated the reference with 'Nelson J, Alvey N, Bowman L, et al. Consensus recommendations for use of maintenance immunosuppression in solid organ transplantation: Endorsed by the American College of Clinical Pharmacy, American Society of Transplantation, and the International Society for Heart and Lung Transplantation. Pharmacotherapy 2022; 42:599-633.'

Comment 4. The technique used to perform therapeutic drug monitoring of tacrolimus is not described. It should be explained.
Reply 4. We apologize for our carelessness. Tacrolimus trough concentrations in the whole blood were measured once daily by the liquid chromatography coupled to tandem mass spectrometry using an Agilent 6460 LC-MS/MS (Agilent Technologies, CA, USA). We added

this information on the technique for TDM in the revised manuscript.

Comment 5. How many blood level determinations were analyzed in each patient? Were any levels discarded? (If affirmative, explain why)
Reply 5. The samples were obtained daily from each patient for the 2 weeks of study period, but no samples were discarded.

Comment 6. There is no description of the statistical methods used by the authors.
Reply 6. This study is an observational study on the tacrolimus moniroting parameters and does not require additional analytic statistics. The descriptive statistics such as calculatins of TTRin, TTRto, CoV have been descrived in the method section.

Comment 7. Line 20: The spelling in the phrase "days in therapeutic range" should be corrected (without the "s" at the end).
Reply 7. We apologize for our carelessness. We corrected the typo in the revised manuscript.

Comment 8. Lines 6-9: This first paragraph is confusing. Please rewrite making clear what was the initial number of patients and what was the final number analyzed, describing the reasons why certain patients were not included.
Reply 8. We apologize for the lack of clarity. During the study period, a total of 74 patients underwent lung transplantation. Of these, 7 patients (3 younger than 18 years old, 3 underwent re-transplantatkion, and 1 died after only one measure of tacrolimus concentration) were exvluded from the study. We modified the sentence to clarify the number of included and excluded patients.

Comment 9. Lines 11-12: The sentences "… the majority (68.7%) were male, although one underwent bilateral lung transplantation (98.5%)" makes no sense. The recipients' sex does not have to be related to the type of transplantation performed (uni- or bipulmonary).
Reply 9. We apologize for our carelessness. The typo of 'although one underwent bilateral lung transplantation…' made you confused. The sentence should have been 'the majority (68.7%) were male, *all but one* underwent bilateral lung transplantation (98.5%).' We fixed the error in the revised manuscript.

Comment 10. Last paragraph: The authors present information regarding observed acute kidney injury following tacrolimus infusion, need for renal replacement therapy, neurotoxicity, and acute rejection. The authors should include in the manuscript (under the methods section) the criteria they used to define each of these variables.
Reply 10. AKI was defined as increase in serum creatinine by 0.3 mg/dl or more within 48 hours, to 1.5 times baseline or more within 7 days, or urine output less than 0.5 ml/kg/h for 6 houres according to the KDIGO. Neurotoxicity was defined as posterior reversible encephalopathy syndrome or minor neurotoxic effects, including headache, tremor, or change in mental status. However, this manuscript is in the form of brief reports, so it was difficult to describe all definitions due to the limited number of word counts. But, we added this definitions in the revised manuscript.

Comment 1. In addition, the authors state that there were no patients who showed acute cellular rejection, could they confirm up to what point the patients were followed up? Because according to the information regarding tacrolimus blood levels, this would only reflect the first 2 weeks after surgery, a time that is not sufficient to evaluate the possible occurrence of acute

rejection.
Reply 11. We thank the reviewer for valuable comments. We totally agree that tacrolimus level during the first two weeks of transplantation would not be associated with the possible occurrence of acute cellular rejection. But, potential readers, including other peer reviewers, would be interested in whether an acute cellular rejection in the early phase of lung transplantation, so we described the results, but it was not related as expected.

Comment 12. Line 22: The authors have mentioned previously that there were no cases of rejection, so it is not possible to state here "the small number of patients and rejection events" as it would be an incongruence.
Reply 12. We intended to describe the small number of enrolled patients and no event of rejection in this study, but typos made a misunderstanding. We modified the sentence according to our results as follow: 'because of the small number of patients and no rejection event'

Comment 13. Page8, Lines 13-21: Did the authors take into consideration that immediate post-transplant variability can be due to factors such as the influence of the surgical intervention itself, concomitant medication after surgery, the patient's condition...? Did they perform any kind of analysis to evaluate whether these factors had an influence on their results?
Reply 13. We thank the reviewer for valuable comments. But, we could not evalauate any factors associated with high variability of the tacrolimus trough concentrations. Drug interaction is most likely, but it is currently difficult to analyze because there are a small number of patients and various drugs. However, this is an important analysis that needs to be done later by adding more patients to our future research topic.

Comment 14. Table 1. The "Total ischemic time" (right and left) is reported. Please explain what influence this may have on tacrolimus level variability and what was observed in this respect in the analyzed sample. Is there any reason for the authors to differentiate between left and right sides? In case this information is not relevant when interpreting the results of this study (related to variability in post-lung transplant tacrolimus levels), this information should be omitted, to avoid leading to confusion.
Reply 14. We simply intended to provide information related to the transplant operation. It is not relevant to the interpretation of our results, so we delete it as you suggested.

Comment 15. There are 16 cases of in-hospital mortality reported, what were the causes?
Reply 15. In most cases, ventilation disorders caused by airway problems (necrosis and stenosis) were the main causes of death.

Reviewer D

This manuscript describes the achievement of therapeutic range of tacrolimus concentration in the first two weeks after lung transplantation using continuous intravenous administration. Compared to other studies in the literature, the authors of this study put their interest not in the clinical outcome but more in the achievement of therapeutic range using continuous intravenous administration. It is an interesting article showing the high variability of tacrolimus range in the first two weeks and describes continues intravenous administration as a safe choice. Overall the manuscript is easily understandable. I find it suitable for a publication after major revision and have the following questions and comments to the authors.
: The authors really appreciate the reviewer's encouraging comments. Regarding to your

comments, we revised our manuscript based on your comments and suggestions.

Comment 1. Administration of basiliximab in every lung transplant recipient is an uncommon approach? Do you use basiliximab as a clinic standard?
Reply 1. Yes, induction with basiliximab is applied as a routine protocol for lung transplantation regardless of the risk for postoperative renal dysfunction or acute rejection.

Comment 2. Can you describe your center standards in more detail? Biopsy in the first 4 weeks? How did you administrate other drugs like corticosteroid and mycophenolate mofetil and in which dose?
Reply 2. Our standard immunosuppressive regimen was based on a triple-drug combination of tacrolimus, mycophenolate mofetil, and corticosteroids. All patients received 500-mg intravenous methylprednisolone before reperfusion, followed by intravenous administration of 0.5 mg/kg for 14 days. Then, we gradually tapered the dose every two weeks to 0.125 mg/kg. When patients are tolerating oral intake, intravenous methylprednisolone was converted oral prednisolone. One 1000-mg mycophenolate mofetil was administered twice daily unless it resulted in leukopenia or liver dysfunction, in which case the dose was lowered or discontinued. In our institute, the surveillance bronchoscopies with transbronchial lung biopsies are not routinely performed, but when indicated by symptoms and sign.

Comment 3. After reaching a steady-state, tacrolimus dosing was switched to an oral equivalent daily dose in patients assured of adequate oral intake. Assuming an oral bioavailability of 10%, the oral equivalent dose was administered twice daily.' Did all patients in this study receive continuous i.v. Tacrolimus during the first two weeks or did some of them receive tarcolimus oral during this time? It is unclear according to this sentence.
Reply 3. Yes, all patietns received tacrolimus with an initial continuous intravenous infusion. As described in the Method section, after reaching a steady-state, tacrolimus dosing was switched to an oral equivalent daily dose in patients assured of adequate oral intake. As described in the result section, steady status was reached and converted to oral form within a median of 6 (5-8) days, and through level was well maintained as shown in Figure 1B. we added this information in the results section.

Comment 4. Line 18: 'All patient records and data were anonymized and de-identified before analysis' Dot at the end of this sentence is missing.
Reply 4. We apologize for our carelessness. We fixed the missing.

Comment 5. Line 6: 'Among them, three fell below 18 years old, three repeated transplantation, and one died after one tacrolimus trough concentration was excluded from the analysis. Can you please reformulate this sentence?
Reply 5. We apologize for the lack of clarity. During the study period, a total of 74 patients underwent lung transplantation. Of these, 7 patients (3 younger than 18 years old, 3 underwent re-transplantatkion, and 1 died after only one measure of tacrolimus concentration) were exvluded from the study. We modified the sentence to clarify the number of included and excluded patients.

Comment 6. Line 12: 'Additionally, the majority (68.7%) were male, although one underwent bilateral lung transplantation (98.5%).' Can you reformulate this sentence?
Reply 6. We apologize for our carelessness. The typo of 'although one underwent bilateral lung transplantation…' made you confused. The sentence should have been 'the majority (68.7%)

were male, *all but one* underwent bilateral lung transplantation (98.5%).' We fixed the error in the revised manuscript.
Comment 7. Line 22: Acute kidney injury following tacrolimus infusion occurred in 23 (31%) patients, with most (18/23, 78%) injuries occurring during the first postoperative week. Is tacrolimus administration the only cause of acute kidney injury or are there other factors like the severity of the operation, haemodynamic changes during and after the operation?
Reply 7. We apologize for the lack of clarity. But, we could not evaluated the tacrolimus related nephrotoxicity itself. As you mentioned, it is difficult to distinguish between tacrolimus-related nephrotoxicity and the various acute kidney injuries that occur after transplantation, so we are simply reporting the frequency of acute kidney injury following tacrolimus infusion.

Comment 8. Furthermore, neurotoxicity of tacrolimus was not observed in patients participating in this study.' You did not observe any neurotoxicity in the first two weeks or also in the further period?
Reply 8. We apologize for the lack of clarity. But, the occurent of neurotoxicity within one month of the postoperative period was considered to be neurotoxicity associated with continuous infusion. We added this information in the method section.

Comment 9. Overall the results section is quite short for this study. It would be better if you describe more about the dosage of tacrolimus. Did you remain the dosage 0.01 mg/kg/day or change it during the first 2 weeks? What was the median level of administered tacrolimus? Did some of the patients received oral tacrolimus during this time?
Reply 9. The dose of tacrolimus during the study period was daily titrated to achieve target of 10-15 ng/ml with daily measure of trough concentration by the clinical pharmacist. In addition, as described in the result section, steady status was reached and converted to oral form within a median of 6 (5-8) days. We added this information in the revised manuscript.

Reviewer E

This report is about the therapeutic range of tacrolimus concentration by intravenous administration after lung transplantation. English is easy to read, however I have some questions for this report.
: The authors really appreciate the reviewer's encouraging comments. Regarding to your comments, we revised our manuscript based on your comments and suggestions.

Comment 1. The authors mentioned the side effect of tacrolimus continuous infusion therapy in the Abstract part. I think the lack of "rejection" at line 16 after acute cellular. Please ensure the phrase "acute cellular".
Reply 1. We apologize for our carelessness. It shoud have been 'acute cellular rejection'. We corrected the typo in the revised manuscript.

Comment 2. I saw Table 1, and the number of bilateral lung transplantation was 66 which may be the number of patients for bilateral lung transplantation. However the authors described "one underwent bilateral lung transplantation (98.5%)" at line 12 page 6. Please confirm the number of patients for bilateral lung transplantation.
Reply 2. We apologize for our carelessness. The typo of 'although one underwent bilateral lung transplantation…' made you confused. The sentence should have been 'the majority (68.7%) were male, *all but one* underwent bilateral lung transplantation (98.5%).' We fixed the error in

the revised manuscript.

Comment 3. I found that 11 patients showed over 15 ug/ml of trough level of tacrolimus on day 1 after lung transplantation from Figure 1A. These were very high trough levels, however the median trough level was 10.02 which was a relatively low level because the authors determined the target range of trough level was 10 to 15. Please describe the reason for the high trough level of the 11 patients. The trough level of tacrolimus is affected by CYP4 coenzyme. For example, the trough level will increase if we use an anti-fungal drug like itraconazole. Are there any reasons why the 11 patients showed high trough levels on day 1 after lung transplantation? In addition, the authors use 0.01mg/kg/day of continuous intravenous infusion dose which was the lowest dose of continuous infusion suggested by Guideline in 1999.
Reply 3. We thank the reviewer for valuable comments. But, we could not evalauate any factors associated with high variability of the tacrolimus trough concentrations. Drug interaction is most likely, but it is currently difficult to analyze because there are a small number of patients and various drugs. However, this is an important analysis that needs to be done later by adding more patients to our future research topic.

Comment 4. I would like to know the duration of the intravenous infusion of tacrolimus after lung transplantation. We usually change the administration from intravenous infusion to oral intake. The result of duration may inform us of the suggestion of how many days we can use intravenous infusion after lung transplantation.
Reply 4. We thank the reviewer for valuable comments. As described in the Method section, after reaching a steady-state, tacrolimus dosing was switched to an oral equivalent daily dose in patients assured of adequate oral intake. As described in the result section, steady status was reached and converted to oral form within a median of 6 (5-8) days, and through level was well maintained as shown in Figure 1B. we added this information in the results section.