Authors' Responses to the Reviewers' Comments

Editor comment: This manuscript has now been evaluated. Preliminary review found that this is a manuscript of meta-analysis that summarized effect of donor hypertension on renal transplant recipients blood pressure, allograft outcomes and survival. The content of this manuscript is potentially fit in the scope of the journal, pending improvement of the English writing, and to follow the journal format, especially reference, etc.

Authors' response: We thank the editor and the reviewers for their kind comments, constructive criticisms and useful suggestions which we have used to improve the quality of this manuscript. We have responded to the issues raised by the editor and reviewers. Changes within the manuscript text are shown in red.

We have previously responded to all the comments by the editor and reviewers. We now include in the present response ALL comments given by each reviewer and how we addressed it

1) Response to Editor comments in the Manuscript

Editor comment: For manuscripts submitted by non-native speakers of English, please provided language certificate by professional English language editing companies.
Authors' response: Thanks, we have provided an English language editing certificate. Alchemist Research and Data Analysis provided language editing services by English speaking medical editor, Dr. Mamta Gupta.

Editor comment: Running title: A short running title of no more than 6 words should be provided. It should state the topic of the paper.
Authors' response: Thanks. I have revised the running title as requested.

Editor comment: Provide missing details in the authors addresses.
Authors' response: Done

Editor comment: Include a section on Research Highlights
Authors' response: Done

Editor comment: Please check and confirm that there are no repeated references!
Authors' response: Done.

All other suggestions given by the editor were closely adhered to.
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2) Response to Reviewer 1 Comments

ALL COMMENTS BY REVIEWER 1 TO AUTHORS

1) please specify what was bleeding % in abstract 2) were the 3 who bled on high dose or low dose NoAC? 3) excellent you looked at pre and post Tacro and sCr levels, can you run a similar analysis for eGFR? as that maybe a better measure of renal function. 4) well done study.

Reviewer comment: 1) please specify what was bleeding % in abstract
Authors' response: Thanks for the suggestion. We have highlighted where we specified this in our Abstract. It reads:

"*No (0%) thromboembolic events were observed during the one-year period, but 3 (7.1%) bleeding events occurred in the cohort consisting of 1 patient treated with rivaroxaban 15mg daily and 2 patients who received apixaban 2.5mg twice daily."*

Reviewer comment: 2) were the 3 who bled on high dose or low dose NoAC?
Authors' response: Thanks for the suggestion. We have included the doses and names of the NOAC agents received by the patients who had bleeding. It reads:

"*No (0%) thromboembolic events were observed during the one-year period, but 3 (7.1%) bleeding events occurred in the cohort consisting of 1 patient treated with rivaroxaban 15mg daily and 2 patients who received apixaban 2.5mg twice daily."*

Reviewer comment: excellent you looked at pre and post Tacro and sCr levels, can you run a similar analysis for eGFR? as that maybe a better measure of renal function.
Authors' response: Done. In line with this suggestion and those of the other reviewers, we have revised this section of the manuscript in the last paragraph of the Results Section. The last paragraph of the Results Section reads:

"*On the other hand, no thromboembolic events (0%) were observed. In addition, no significant change in serum tacrolimus level was observed three days after the initiation of NOACs among patients treated with tacrolimus (pre- and post-NOACs serum tacrolimus level was 7.25 and 7.89 ng/mL, p = 0.55). Similarly, after one year of treatment with NOACs there was no significant change in the pre- and post-NOACs serum creatinine level with mean levels of 107.6µmol/L and 113.11µmol/L (p=0.772) respectively, (median 107.5 vs. 108.5µmol/L, respectively). This is summarized in Figure 1. Besides, as shown in Figure 2, pre- and post-NOACs eGFR levels after one-year of treatment with NOACs did not significantly change with respective mean levels of 72.2ml/min/1.73m^2 and 65.9ml/min/1.73m^2 (p = 0.232; median: 68.2 vs. 60.4ml/min/1.73m^2, respectively)."*

3) Response to Reviewer 2 Comments

ALL COMMENTS BY REVIEWER 2 TO AUTHORS

The manuscript may be the first study addressing the efficacy and safety of NOACs. Thus, it adds new information to the field. It would be better if the authors can emphasize the advantage of NOACs in comparison to the other drugs; no effect on kidney function (especially in "discussion"). In that way, audience can realize the value of the study better.
• Minor grammatical errors such as "…study that addresses…" (page 10). Please thoroughly check the text.

Reviewer comment: The manuscript may be the first study addressing the efficacy and safety of NOACs. Thus, it adds new information to the field. It would be better if the authors can emphasize the advantage of NOACs in comparison to the other drugs; no effect on kidney function (especially in "discussion"). In that way, audience can realize the value of the study better.
Authors' response: We thank the reviewer for their kind comments, constructive criticisms and useful suggestions which we have used to improve the quality of this manuscript. We have responded to the issues raised by the editor and reviewers. Changes within the manuscript text are shown in red.

We have extensively revised the Discussion section in line with the suggestions of this reviewer and other reviewers. We wish to point out that in the revised manuscript we have extensively discussed the advantages of NOACS, its drug-drug interactions and Limitations in the Discussion section. The additions in the Discussion section now reads:

"*In our study, we didn't report any thromboembolic event in any of the patients after CNI initiation. This might suggest NOACs are as effective in kidney transplantation population as the general population. Also, we had a few bleeding events with low doses (2.5mg twice daily) of apixaban and a moderate dose (15mg daily) of rivaroxaban, which may suggest a good safety profile. However, there is a need to further assess the mechanisms of bleeding in patients exposed to NOACs. Although our study indicates that NOACs may be safe and effective for the prevention and treatment of thromboembolic events in renal transplant recipients, there is a need to highlight some of its important advantages and disadvantages compared to other vitamin K antagonists. Its major advantages include absence of food interactions, few strong drug interactions, predictable pharmacokinetic and pharmacodynamic properties, a rapid onset and offset of action, a short half-life, and the absence of the need for laboratory monitoring.*[13]

However, pharmacokinetic and pharmacodynamic studies show that NOACs elimination is dependent on renal clearance to varying extents; but compared with vitamin K antagonists, the efficacy and safety of the NOACs is preserved in patients with moderate renal impairment.[14-15] *There is a need to administer NOACs with caution in individuals with severe kidney or*

hepatic damage particularly the elderly. This is because up to 25%, 33% and 80% of apixaban, rivaroxaban and dabigatran, respectively are eliminated through the kidneys as an active drug.[13-15] In severe renal or hepatic damage, the elimination of the drug may be affected requiring adjustments in the dosing of the NOAC agent.

Our analysis only included renal transplant recipients with an eGFR of >54 ml/min/1.73m2. Therefore, dosage adaptation of the NOACs should ideally not be necessary. However, considering the very limited or no prior experience in the use of NOACs in kidney transplant recipients (with/without renal impairment), doses of NOACs were administered to the patients in this study using the Health Canada dosing algorithm for each of the NOACs according to renal function and clinical status of the patients.[14,16] Thus, the effectiveness of NOACs observed in our data can only be interpreted in the context of kidney transplant recipients with sufficiently preserved renal function. Several clinical trials such as the EINSTEIN, ARISTOTLE, and RE-LY trials have previously demonstrated the safety and efficacy of these NOACs in individuals with varying levels of renal impairment.[17-19]

In the present study, 3 of the subjects received dabigatran with tacrolimus-based CNIs. Previous studies have called for caution in the use of NOACs and immunosuppressive agents due to the potential for drug-drug interactions.[8,20-21] A study suggested that dabigatran should not be administered to patients receiving CNIs because CNIs are known substrates of both CYP 450 3A4 and P-gp, and can lead to increased exposure to dabigatran.[8,20] Because of the limited evidence of NOACs usage with CNIs in the setting of solid organ transplantation, this clinical recommendation was made based on an underpowered analysis of nine heart transplant recipients immunosuppressed with CNIs and treated with dabigatran for atrial fibrillation, VTE, or atrial thrombus.[8] In the study, patients who received tacrolimus with dabigatran were more likely to require a decrease in tacrolimus dose during therapy and numerically had more major bleeding events.[8] However, observations from the RE-LY trial indicate that concomitant use of dabigatran with P-gp inhibitors (like amiodarone or verapamil) increased dabigatran exposure but was not associated with significant differences in the event rate or bleeding.[22-23] A recent review indicates that in patients receiving dabigatran etexilate for the treatment and prevention of VTE, there is no need for dose adjustments and no contraindication to its co-administration with P-gp inhibitors so long as the patients have a creatinine clearance greater than 50 mL/min.[24] All the patients in our study had creatinine clearance greater than 50 mL/min and none of those who received dabigatran had a bleeding event. Recent expert opinion conclude that provided adequate attention is given to renal function, the co-administration of NOACs and CNIs in solid organ transplantation is safe and effective.[24]

This study has some limitations. First, this was a retrospective observational study, therefore any reported association does not imply causation. Second, all the patients in this study had sufficiently preserved renal function (creatinine clearance >50 mL/min), therefore we cannot report on the safety or efficacy of the NOACs in kidney transplant recipients with substantial renal impairment. Third, more than half of the patients received low doses of the NOAC agent. Therefore, our finding may not reflect the outcomes in renal transplant recipients treated with

higher doses of NOAC agent.

In conclusion, our study suggests that NOACs may be safe and effective for the prevention and treatment of thromboembolic events in renal transplant recipients with limited complications. Further studies need to be conducted to assess the effectiveness and safety profile of NOACs compared to other vitamin K antagonists (e.g. warfarin) in kidney transplant population."

Reviewer comment: Minor grammatical errors such as "…study that addresses…" (page 10). Please thoroughly check the text.
Authors' response: Thanks. We have reviewed the text and performed all other grammatical corrections as necessary.

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4) Response to Reviewer 3 Comments

ALL COMMENTS BY REVIEWER 3 TO AUTHORS

The present manuscript reports a retrospective analysis of the efficacy and safety of NOACs after kidney transplantation. Date are presented on 42 patients who received either apixaban, rivaroxaban or dabigatran for either atrial fibrillation or thromboembolism and were followed for 1 year. In general, the data on NOACs in kidney transplant patients bear novelty, however the data presentation and interpretation needs attention. - The analysis only includes patients with a eGFR of >54. Therefore, no dosage adaptation should be necessary for NOACs. This is also why the data can only be interpreted in terms of sufficiently preserved kidney function. This has to be mentioned in the discussion section and has to be compared to published data. - Why did you use only 15mg of rivaroxaban instead of 20mg although the GFR was >50ml/min? - Is is not understandable why the patients were subdivided according to age extensively. It would make more sense to compare patients <75/≥75 years of age since this is 1) relevant for indication of oral anticoagulation and 2) may have an impact on the NOAC dosage. - In addition, it is very important to clearly indicate wich NOAC was used with what kind of immunosuppression. - Did the patients also receive MMF and/or steroids? Please indicate. - The analysis does not have a group for comparison. It would extremely strenghthen the analysis if the authors would add a matched cohort of kidney transplant recipients on vitamin K antagonists. - The use of dabigatran together with CNIs is contraindicated due to P-gp-induction leading to increased exposure to dabigatran. Did you use dabigatran together with CNIs? Please clarify. - The discussion section is extremely short and lacks study limitations. Please discuss possible interactions with immunosuppression, limitations of your study and the current literature in view of your data in more detail. - Table 2: please specify „antiplatelet" Abstract: - „The main indications for NOACs use in the cohort were atrial fibrillation 25 (59.5%) and venous thromboembolism 10 (23.8%)." – should be changed to

„… were atrial fibrillation in 25 patients (59.5%)…“ in the abstract and the whole manuscript throughout. - „There were no significant changes in the pre- and post-NOACs tacrolimus (p = 0.55), creatinine (p > 0.05) and eGFR levels (p >0.05).“ – The meaning of this sentence is unclear. - The abstract should include information on the time period since kidney transplant (and also the main text), on median GFR prior initiation of NOACs and after 1 year of NOACs. Language polishing necessary.

Reviewer comment: 1) The present manuscript reports a retrospective analysis of the efficacy and safety of NOACs after kidney transplantation. Date are presented on 42 patients who received either apixaban, rivaroxaban or dabigatran for either atrial fibrillation or thromboembolism and were followed for 1 year. In general, the data on NOACs in kidney transplant patients bear novelty, however the data presentation and interpretation needs attention.
Authors' response: We thank the reviewer for their kind comments, constructive criticisms and useful suggestions which we have used to improve the quality of this manuscript. We have responded to the issues raised by the editor and reviewers. Changes within the manuscript text are shown in red.

Below is the point-by-point response to the comments given by this reviewer.

Reviewer comment: The analysis only includes patients with a eGFR of >54. Therefore, no dosage adaptation should be necessary for NOACs. This is also why the data can only be interpreted in terms of sufficiently preserved kidney function. This has to be mentioned in the discussion section and has to be compared to published data.
Authors' response: We thank the reviewer for the comments. We agree with the reviewer that our data included patients with eGFR of >54 and we need to highlight that the data can only be interpreted in terms of sufficiently preserved kidney function.

In line with the comment of this and other reviewers, we have highlighted that our study patients had eGFR of >54 and therefore the outcomes of this study is for renal transplant recipients with sufficiently preserved kidney function. We have also identified few related literatures to support our discussion.

However, because we could not find any prior study on the use of NOACs in renal transplant recipients, we could not find any published data in this group of patients to compare our findings.

The additional paragraphs now read:

"*Although our study indicates that NOACs may be safe and effective for the prevention and treatment of thromboembolic events in renal transplant recipients, there is a need to highlight some of its important advantages and disadvantages compared to other vitamin K antagonists. Its major advantages include absence of food interactions, few strong drug interactions,*

predictable pharmacokinetic and pharmacodynamic properties, a rapid onset and offset of action, a short half-life, and the absence of the need for laboratory monitoring.[13]

However, pharmacokinetic and pharmacodynamic studies show that NOACs elimination is dependent on renal clearance to varying extents; but compared with vitamin K antagonists, the efficacy and safety of the NOACs is preserved in patients with moderate renal impairment.[14-15] There is a need to administer NOACs with caution in individuals with severe kidney or hepatic damage particularly the elderly. This is because up to 25%, 33% and 80% of apixaban, rivaroxaban and dabigatran, respectively are eliminated through the kidneys as an active drug.[13-15] In severe renal or hepatic damage, the elimination of the drug may be affected requiring adjustments in the dosing of the NOAC agent.

Our analysis only included renal transplant recipients with an eGFR of >54 ml/min/1.73m2. Therefore, dosage adaptation of the NOACs should ideally not be necessary. However, considering the very limited or no prior experience in the use of NOACs in kidney transplant recipients (with/without renal impairment), doses of NOACs were administered to the patients in this study using the Health Canada dosing algorithm for each of the NOACs according to renal function and clinical status of the patients.[14,16] Thus, the effectiveness of NOACs observed in our data can only be interpreted in the context of kidney transplant recipients with sufficiently preserved renal function. Several clinical trials such as the EINSTEIN, ARISTOTLE, and RE-LY trials have previously demonstrated the safety and efficacy of these NOACs in individuals with varying levels of renal impairment.[17-19]"

Reviewer comment: Why did you use only 15mg of rivaroxaban instead of 20mg although the GFR was >50ml/min?
Authors' response: We thank the reviewer for the comments. We have added this clarification in our Discussion. It reads

"*considering the very limited or no prior experience in the use of NOACs in kidney transplant recipients (with/without renal impairment), doses of NOACs were administered to the patients in this study using the Health Canada dosing algorithm for each of the NOACs according to renal function and clinical status of the patients [14,16].*"

Reviewer comment: - Is is not understandable why the patients were subdivided according to age extensively. It would make more sense to compare patients <75/≥75 years of age since this is 1) relevant for indication of oral anticoagulation and 2) may have an impact on the NOAC dosage.
Authors' response: We thank the reviewer for the comments. We have revised Table 2 in line with the suggestion of the reviewer and compared patients who were <75 years old versus those older than ≥75 years of age.

Reviewer comment: The analysis does not have a group for comparison. It would extremely strenghthen the analysis if the authors would add a matched cohort of kidney transplant recipients on vitamin K antagonists..
Authors' response: We thank the reviewer for the comments. This is a retrospective study, we did not have a "matched cohort" to compare our subjects with. However, given the very limited evidence in this field we believe our findings will be an important addition to the literature.

Reviewer comment: The use of dabigatran together with CNIs is contraindicated due to P-gp-induction leading to increased exposure to dabigatran. Did you use dabigatran together with CNIs? Please clarify. -. Please discuss possible interactions with immunosuppression,
Authors' response: We thank the reviewer for the concern raised. We have added a paragraph extensively discussing the possible interactions between NOACs and immunosuppression in transplant recipients. It reads:

"In the present study, 3 (7.2%) of the subjects received dabigatran with tacrolimus-based CNIs. Previous studies have called for caution in the use of NOACs and immunosuppressive agents due to the potential for drug-drug interactions.[8,20-21] A study suggested that dabigatran should not be administered to patients receiving CNIs because CNIs are known substrates of both CYP 450 3A4 and P-gp, and can lead to increased exposure to dabigatran.[8,20] Because of the limited evidence of NOACs usage with CNIs in the setting of solid organ transplantation, this clinical recommendation was made based on an underpowered analysis of nine heart transplant recipients immunosuppressed with CNIs and treated with dabigatran for atrial fibrillation, VTE, or atrial thrombus.[8] In the study, patients who received tacrolimus with dabigatran were more likely to require a decrease in tacrolimus dose during therapy and numerically had more major bleeding events.[8] However, observations from the RE-LY trial indicate that concomitant use of dabigatran with P-gp inhibitors (like amiodarone or verapamil) increased dabigatran exposure but was not associated with significant differences in the event rate or bleeding.[22-23] A recent review indicates that in patients receiving dabigatran etexilate for the treatment and prevention of VTE, there is no need for dose adjustments and no contraindication to its co-administration with P-gp inhibitors so long as the patients have a creatinine clearance greater than 50 mL/min.[24] All the patients in our study had creatinine clearance greater than 50 mL/min and none of those who received dabigatran had a bleeding event. Recent expert opinion conclude that provided adequate attention is given to renal function, the co-administration of NOACs and calcineurin inhibitors in solid organ transplantation is safe and effective.[24]"

Reviewer comment: Please clarify. - The discussion section is extremely short and lacks study limitations. Please discuss possible limitations of your study and the current literature in view of your data in more detail.
Authors' response: We have extensively expanded the Discussion of our findings (above) and added a paragraph on Study Limitations. The Limitations section reads

It reads:
"*This study has some limitations. First, this was a retrospective observational study, therefore any reported association does not imply causation. Second, all the patients in this study had sufficiently preserved renal function (creatinine clearance >50 mL/min), therefore we cannot report on the safety or efficacy of the NOACs in kidney transplant recipients with substantial renal impairment. Third, more than half of the patients received low doses of the NOAC agent. Therefore, our finding may not reflect the outcomes in renal transplant recipients treated with higher doses of NOAC agent*."

Reviewer comment: - Did the patients also receive MMF and/or steroids
Authors' response: Thank you very much. Yes they all received both oral prednisolone and mycophenolate mofetil. We have reflected this in the manuscript

Reviewer comment: Table 2: please specify „antiplatelet"
Authors' response: We have provided the name of the antiplatelet used as recommended

Reviewer comment: In addition, it is very important to clearly indicate wich NOAC was used with what kind of immunosuppression
Authors' response: We have provided an additional Table (Table 3) which clearly indicate which NOAC agent was used with what kind of immunosuppression as recommended by the reviewer. Please, see new table 3

Reviewer comment: The abstract should include information on the time period since kidney transplant (and also the main text), on median GFR prior initiation of NOACs and after 1 year of NOACs.
Authors' response: We have included the time period since kidney transplant. However, median GFR and creatinine clearance prior to the initiation of NOACs and after 1 year of NOACs were not included in the Abstract as suggested as the Abstract is now too wordy (About 400 words).

Reviewer comment: Abstract: - „The main indications for NOACs use in the cohort were atrial fibrillation 25 (59.5%) and venous thromboembolism 10 (23.8%)." – should be changed to „… were atrial fibrillation in 25 patients (59.5%)…" in the abstract and the whole manuscript throughout. - „
Authors' response: We have made these revisions in line with the reviewer's suggestions

Reviewer comment: „There were no significant changes in the pre- and post-NOACs tacrolimus ($p = 0.55$), creatinine ($p > 0.05$) and eGFR levels ($p > 0.05$)." – The meaning of this sentence is unclear. -
Authors' response: We have now revised this aspect of our Results section to be clearer. It is reflected in the last paragraph of the Results Section. It now reads:

"On the other hand, no (0%) thromboembolic events were observed. In addition, no significant change in serum tacrolimus level was observed three days after the initiation of NOACs among

patients treated with tacrolimus (pre- and post-NOACs serum tacrolimus level was 7.25 and 7.89 ng/mL, p = 0.55). Similarly, after one year of treatment with NOACs there was no significant change in the pre- and post-NOACs serum creatinine level with mean levels of 107.6µmol/L and 113.11µmol/L (p=0.772) respectively, (median 107.5 vs. 108.5µmol/L, respectively). This is summarized in Figure 1. Besides, as shown in Figure 2, pre- and post-NOACs eGFR levels after one-year of treatment with NOACs did not significantly change with respective mean levels of 72.2ml/min/1.73m^2 and 65.9ml/min/1.73m^2 (p = 0.232; median: 68.2 vs. 60.4ml/min/1.73m^2, respectively)".

Reviewer comment: The abstract should include information on the time period since kidney transplant. -(and also the main text), on median GFR prior initiation of NOACs and after 1 year of NOACs.
Authors' response: We have now revised the Abstract to include the time period since kidney transplant and other requested details. The Results section of the Abstract now reads:

"Complete data on 42 renal transplant patients were retrieved: 59.5% males, 90.5% were whites and 66.7% were older than 60 years old. The mean duration since renal transplantation of the patients was 8.8 ± 7.4 years. The most common risk factors for the development of end-stage renal disease in the subjects were hypertension (19.0%), polycystic kidney disease (19.0%), followed by diabetic nephropathy (16.7%) and chronic glomerulonephritis (16.7%). The main indications for NOACs use in the cohort were atrial fibrillation in 25 patients (59.5%) and venous thromboembolism in 10 patients (23.8%). Overall, 29 patients (69%) were treated with apixaban, 10 patients (23.8%) with rivaroxaban and 3 patients (7.14%) with dabigatran. No (0%) thromboembolic events were observed during the one-year period, but 3 (7.1%) bleeding events occurred in the cohort consisting of 1 patient treated with rivaroxaban 15mg daily and 2 patients who received apixaban 2.5mg twice daily. There were no significant changes in serum tacrolimus level three days after the initiation of NOACs among patients treated with tacrolimus (pre- and post-NOACs tacrolimus levels were 7.2516 and 7.8867 ng/mL, p = 0.55, respectively). Also, after one-year of treatment with NOACs there were no significant changes in the pre- and post-NOACs serum creatinine level (p=0.772) and estimated glomerular filtration rates (p=0.232)."

Reviewer comment: Language polishing necessary.
Authors' response: We have now revised the manuscript generally and did language polishing as necessary throughout the paper

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We thank all the reviewers for their kind comments, constructive criticisms and useful suggestions which we have used to improve the quality of this manuscript. We hope we have addressed all the issues raised by the reviewers. We will be happy to respond to any other issues the reviewers may deem necessary.

Many thanks once again.