

Manuscript No: 56804

Title: The role of novel biomarkers in kidney transplantation

Dear Editor and reviewers,

Thank you for the careful review of our manuscript. As a result of your thoughtful comments and critique, the quality of the paper has improved significantly. Please find our point-by-point response below.

Specific Comments to Authors:

Reviewer #1

The authors present a very well organized review regarding biomarkers use in the context of key kidney transplant outcomes

--Thank you

Plasma-derived fractalkine should mention in brackets (CX3CL1) so the readers can understand that it belongs to chemokine C-X-C motif group.

--Change made and included on page 3 in the Abbreviations section as well as page 14, line 4.

In page 13, line 7, I believe there is a typo: (AUC (0.866) should be (AUC = 0.866).

- *Sorry about that, change made to (AUC = 0.866); now on page 14 line 7.*

In page 15, line 6, please review the number of biopsies because the clinical judgment agreement of 87% and histology (80%) ($p = 0.0042$) regards to 451 biopsies and not 519. –

- *Thank you for pointing that, change made to the number of biopsies to 451 from 519 now on page 16 line 7.*

TruGraf is registered trademark and it should be cited as TruGraf®

--All mentions of TruGraf® updated to include the registered trademark.

In page 18 it's the first time GEP is mentioned and should explained

--GEP explained on page 19, line 21.

In page 20 it's the first time CMS is mentioned and should explained

-- Centers for Medicare & Medicaid Services (CMS) included in abbreviation on page 2, line 23 and defined on page 22, line 7

In page 21 the P values mentioned omits the zero to the left of the comma

- *Sorry about that, P values updated to include zero preceding decimal point, now on page 22.*

In page 22 DART is used before explained (although explained just after)

-- Circulating Donor-Derived Cell-Free DNA in Blood for Diagnosing Acute Rejection in Kidney Transplant Recipients (DART) defined on page 3, line 11, and described on page 23, lines 18 and 19.

Allosure is registered trademark and it should be cited as Allosure® in Page 22

--Allosure® labeled with registered trademark when referenced

In page 24 DGF it's the first time GEP is mentioned and should explained

--Gene expression profile (GEP) listed in abbreviations on page 4, line 10, and defined on page 20.

In page 26 the number KTr evaluated should be reviewed; I believe the final study population of 1027 and not 2207.

--Corrected to study population of 1027 in all mentions, specifically page 31.

In page 28 please review the polymorphism designation NFKB1-94ins/ins; should it be - 94ins/delATTG?

--Changed to 94ins/delATTG now on page 35.

Other consideration: - Do the authors think that Bk virus should be mentioned in the Infection sub-section?

--Excellent suggestion. Novel biomarkers in the context of BK is added in page 35-37.

What about covid-19 pneumonia infection?

--Another great suggestion. COVID19 section included from page 37-38.

Specific Comments to Authors:

Reviewer #2

This is a well written review on a topic that remains of interest, although finding an ideal biomarker that is of high sensitivity and specificity for a specific outcome measure of kidney transplantation remains similar to finding the "Holy Grail". I like the approach of the authors in their review dividing biomarkers into immunological and non-immunological, and then analysing biomarkers for specific conditions/outcomes, and it makes sense to review novel data from the last five years.

--Thank you for your nice words.

However, I am missing two major domains in the review:

- A. biomarkers in preservation solution (predominantly relating to DGF), below a few examples of key references (but not systematically searched) 1. Van den Akker EK et al. Eur Surg Res. 2015 Dec;55(4):319-327.(NGAL and DGF) 2. Roest HP et al. Cell free miR-505-3p in preservation solution and DGF. Transplantation. 2019 Feb;103(2):329-335. 3. Van Balkom B et al. Proteins in preservation fluid and DGF. Clin J Am Soc Nephrol. 2017 May 8;12(5):817-824

--Thank you for the suggestion! We incorporated biomarkers in the preservation solution related to DGF within the DGF section on starting on page 30.

- B. biomarkers in machine preservation of kidneys (predominantly to assess graft quality): 1. Weissenbacher A et al AJT 2019 2. Van Smaalen TC et al. Transplantation 2017 3. Parikh CR et al. AJT 2016 4. Hamaoui et al. J Surg Res 2017 5. De Beule/Jochmans. J Clin Med. 2020 Mar 23;9(3). 6. Moser MAJ. Ann Transplant 2017 7. Guzzi F et al. Transplant Int 2020. I really think these areas should be covered, and will make the review excellent!

--Thank for the recommendation. We have added a new section of "Graft quality" specifically on machine preservation starting on page 26. We included these and other references.

Also, some other relevant review papers on the topic should be incorporated and referenced to: 1. Jamshaid Fet al. Int J Clin Pract. 2018 Jul 16:e13220. 2. Van den Akker EK et al. J Transplant. 2015;2015:354826. 3. O'Callaghan J et al. Curr Opinion Organ Transplant 2019 4. Knight SR et al. Transplantation 2019

--Appreciate the additional references. We have Included all those references as suggested.

Looking forward to seeing the revised MS.

--Thank you! We hope we were able to answer all your questions.

Science Editor/Editorial Office's comments and suggestions:

Summary of the Peer-Review Report: The authors presented a very well organized review regarding biomarkers use in the context of key kidney transplant outcomes.

--Thank you.

However, biomarkers in preservation solution or machine preservation of kidneys were missed, the authors should add them and the related references.

-- Biomarkers in preservation solution/machine preservation now included with related references.

The questions raised by the reviewers should be answered

--Reviewers' questions answered.

Issues raised: PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references.

--PMID and DOI numbers included in the reference list.