

**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.*