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RESEARCH ARTICLE

REVISED Measurement of a 5-gene panel in whole blood in kidney transplant recipients with acute rejection and stable controls [version 2; peer review: 1 approved, 1 approved with reservations]

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v2 First published: 28 Sep 2015, 4:913
<https://doi.org/10.12688/f1000research.6941.1>
 Latest published: 12 Apr 2016, 4:913
<https://doi.org/10.12688/f1000research.6941.2>

Abstract

New biomarkers are required to detect acute rejection (AR) in kidney transplant recipients (KTRs) to avoid invasive kidney biopsies. We assess whether a 5-gene panel (*DUSP1*; *NKTR*; *PBEF1*; *MAPK9*; and *PSEN1*) in whole blood samples that has previously been shown to identify AR in a paediatric KTR population is able to distinguish AR in a UK population of adult Caucasian KTR patients.

Keywords

Rena, allograft, rejection, biomarker, acute, whole blood, kidney, gene expression

Open Peer Review**Approval Status** ? ✓

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version 2

(revision)

12 Apr 2016

version 1

28 Sep 2015

view

view

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Any reports and responses or comments on the article can be found at the end of the article.

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Competing interests: No competing interests were disclosed.

Grant information: Wellcome Trust grant 082519/Z/07/Z awarded to Professor Kathryn Wood funded this work.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Betts G, Van Der Net J, Shankar S *et al.* **Measurement of a 5-gene panel in whole blood in kidney transplant recipients with acute rejection and stable controls [version 2; peer review: 1 approved, 1 approved with reservations]**

F1000Research 2016, 4:913 <https://doi.org/10.12688/f1000research.6941.2>

First published: 28 Sep 2015, 4:913 <https://doi.org/10.12688/f1000research.6941.1>

REVISED Amendments from Version 1

This update version contains more data in the conclusions as requested by reviewers. We make it clear that the patients studied in our research did not have donor specific antibodies (DSA), in contrast to those studied by Li *et al.*

In our conclusion we also draw more attention to the small sample size but argue for the possibility of incorporating our data in to future studies.

See referee reports

Introduction

Further improvements are required to monitor rejection of kidney allografts post-transplant. Current methodology to detect rejection by kidney biopsy is invasive and presents a risk to the patient, whilst an inadequate biopsy sample may prevent an accurate diagnosis. The patient risk associated with biopsy deters some transplant centers from performing protocol biopsies, instead using clinical signs of allograft damage to inform a decision to perform an indication biopsy. Significant immune mediated damage to the allograft may occur before rejection is clinically visible. Therefore new biomarkers that can be repeatedly measured by minimally invasive sampling that identifies damaging alloreactivity prior to significant pathology and assesses the whole organ are required¹.

A 5-gene panel (*DUSP1*; *NKTR*; *PBEF1*; *MAPK9*; and *PSEN1*) in whole blood has been shown to distinguish stable allografts from those with acute rejection (AR) in paediatric kidney transplant recipients (KTRs) with mixed ethnicity. Expression of each gene was significantly different between kidneys with AR vs stable function in a separate paediatric validation population (of which 60% had received a deceased-donor allograft)². Interestingly, expression of these genes appeared to identify borderline rejection as AR and may therefore predict AR before it has become clinically evident.

The same panel was recently validated in a Korean adult KTR population, although only *PSEN1* and *MAPK9* were significantly different between AR and control groups³. The lack of differential expression between stable and AR groups for genes *DUSP1*, *NKTR* and *PBEF1* might demonstrate a variability in gene expression between KTR populations; however these three genes did contribute to the ability of the 5-gene panel to distinguish AR from non-AR using multivariate logistic regression analysis.

The expression data presented in our study suggests that these 5 genes, measured in whole blood of KTRs, do not distinguish between stable function and AR in Caucasian KTRs in the UK. Our data, provided with complete clinical and raw gene expression data, will be available to be incorporated into larger studies in the future, providing an important resource.

Methods

7 KTRs with clinical evidence of AR confirmed by allograft biopsy (5 AR, 2 borderline changes suspicious for AR) and 5 control KTRs with stable graft function closely matched for Human Leukocyte Antigen mismatch, race, initial graft function, induction and maintenance therapy were studied (Supplementary material Table 1;

REC number 07/H0603/26, following informed consent). KTRs were adult Caucasians of which 20% had received a deceased-donor allograft. All patients were negative for donor specific antibodies (DSA) pre-transplantation and therefore did not receive desensitization. There was no difference in panel reactive antibodies between control and AR groups. Blood samples were collected during AR episodes occurring on days 4, 6, 6, 41, 48, 210 and 393 in individual KTRs post transplant.

cDNA was produced from whole blood RNA collected in BD tempus tubes and isolated using the Tempus Spin RNA Isolation kit (BD). Expression of a 5-gene panel comprising *DUSP1*, *PBEF1*, *PSEN1*, *MAPK9*, *NKTR* (normalized with *HPRT* expression; Supplementary material Table 2) was accessed by qPCR using TaqMan Gene Expression Assays (Life Technologies) and measured using a Stratagene Mx3000P qPCR machine (Agilent Technologies). Kits were used according to manufacturers instructions.

Each AR episode was treated successfully with three i.v. daily doses of 500-mg methylprednisolone, as measured by restored eGFR to baseline. Two patients with AR additionally received 30mg i.v. alemtuzumab. All patients with AR episodes were re-bled at 1 year post-transplantation, and maintained stable graft function and eGFR, except 1 KTR with AR at day 393 and a second patient that declined follow-up at 1 year.

Univariate logistic regression analysis was performed using IBM SPSS version 22. All other statistics were performed in GraphPad Prism version 5.0c.

Results

Univariate logistic regression analysis was performed on $2^{-\Delta\Delta C_t}$ values calculated using either the pre-operative time point ΔC_t of each KTR⁴ (Figure 1; *DUSP1*: $p=0.772$; *MAPK9*: $p=0.733$; *PBEF1*: $p=0.525$; *NKTR*: $p=0.698$; *PSEN1*: $p=0.935$) or an average of all stable KTR ΔC_t values as a calibrator for each gene. With both methods of $2^{-\Delta\Delta C_t}$ calculation, univariate logistic regression analysis showed that no single gene was a significant predictor of AR. The size of our cohort was insufficient to allow for multivariate analysis. Expression of each gene showed no significant difference when AR and one year samples (*DUSP1*: $p=0.44$; *MAPK9*: $p=0.44$; *PBEF1*: $p=0.44$; *NKTR*: $p=0.31$; *PSEN1*: $p=0.44$) or AR and stable KTR (*DUSP1*: $p=0.88$; *MAPK9*: $p=0.76$; *PBEF1*: $p=0.76$; *NKTR*: $p=0.52$; *PSEN1*: $p=1.0$) samples were compared using Wilcoxon matched-pairs signed rank test and Mann-Whitney test respectively.

Conclusion

This study indicates that the expression of this 5-gene panel is unable to distinguish AR from stable allograft function in this adult Caucasian population, which might be explained by differences in expression of these genes between different populations. It is important to note that Li *et al* demonstrated a change in gene expression only occurred in the presence of DSA and the patients examined in our study were negative for DSA. Importantly, the raw data provided in supplemental tables here are available to supplement future studies involving larger cohorts of patients. We acknowledge that the number of patients in this study is small and

Figure 1. ΔCt values were calibrated using each patient's pre-operative sample ΔCt value to produce $2^{-\Delta\Delta Ct}$ values, shown here and depicting the mean and SEM.

statistical analysis of the cohort is limited by this fact; however we believe this offers an important preliminary examination of the efficacy of using these markers to identify acute rejection in an adult Caucasian KTR population. The availability of our anonymized patient clinical information and raw data, including Ct values for all genes and endogenous control makes this study an important source to combine into a larger cohort of patients to further examine the validity of these markers to identify acute rejection in adult Caucasian KTRs.

Author contributions

Gareth Betts: performed experiments, analysed data, wrote manuscript; Jeroen Van Der Net: identified and analysed clinical details of patients; Sushma Shankar: identified and analysed clinical details of patients; Peter Friend: designed research, intellectual input;

Paul Harden: intellectual input, analysis of clinical details of patients; Kathryn Wood: designed research, wrote grants that provided funds to perform work, intellectual input.

Competing interests

No competing interests were disclosed.

Grant information

Wellcome Trust grant 082519/Z/07/Z awarded to Professor Kathryn Wood funded this work.

I confirm that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgement

The authors thank Sally Ruse for phlebotomy and organizing patient logistics.

Supplementary material

Table 1. Full clinical details of KTRs with an acute rejection episode and stable controls is shown. Columns A–F represent patient demographics; columns G–K initial immunosuppression; column L initial graft function; columns M–Q biopsy data; column R long-term outcomes.

[Click here to access the data.](#)

<http://dx.doi.org/10.5256/f1000research.6941.s103109>

Table 2. Gene expression Ct values for *DUSP1*, *MAPK9*, *PBEF1* (*NAMPT*), *NKTR*, *PSEN1* and the endogenous control *HPRT*. Data was collected in duplicate tubes and the average gene expression was determined. The average of *HPRT* expression was subtracted from the average gene expression of the target gene, to create Δ Ct values for target genes. $\Delta\Delta$ Ct values were subsequently derived by subtracting Δ Ct of the preoperative time point from the Δ Ct of the time point of interest: e.g. (AR time point Δ Ct *DUSP1*) – (preoperative time point Δ Ct *DUSP1*) = $\Delta\Delta$ Ct*DUSP1*. $\Delta\Delta$ Ct values were expressed as $2^{-\Delta\Delta\text{Ct}}$ values.

[Click here to access the data.](#)

<http://dx.doi.org/10.5256/f1000research.6941.s103110>

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Version 1

Reviewer Report 08 March 2016

<https://doi.org/10.5256/f1000research.7474.r12321>

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This is valuable work that has the caveat that it is applicable to Caucasian recipients. That findings were easily translatable from a pediatric population to adults implies some robustness. This needs to be validated in further study.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 14 October 2015

<https://doi.org/10.5256/f1000research.7474.r10625>

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Overall, an important preliminary study assessing the 5 gene panel in whole blood to distinguish acute rejection from stable kidney allografts. The study is very small of only 7 AR (and 2 are borderline AR), and 5 controls, so it is unclear whether there is statistical power to generate the significant differences observed in previous studies referenced as #2 and #3. Inclusion of a discussion on the limitation of this study would be useful.

There is a discrepancy with the study by Li *et al* 2012, however Li *et al.* indicated "all 5 genes had significant change in expression only with the presence of donor specific antibody" and since the 7 patients did not have DSA, this difference should be highlighted.

With regards to the Lee *et al.* 2014 study, the overall discriminatory effectiveness of the 5 gene set was observed to be much reduced compared to the Li *et al* study. Only MAPK9 and PSEN1 were significantly different in AR, even then there was wide overlap in the values between the CMR and Healthy control groups. Notably there was no difference in the gene expression between the AMR and healthy control groups, a note of concern since the Li *et al.* demonstrated an association between DSA and the 5 gene panel.

Furthermore it is unclear how the raw data could be used to supplement future studies; clarification is therefore needed. Without such a road map for the potential use of the data set, the study may be too preliminary for firm conclusions.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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