BENEFICIAL IMMUNOMODULATORY EFFECT OF CHOLECALCIFEROL REPLETION IN CKD PATIENTS : DOWN-REGULATION OF CELL SURFACE HLA-DR EXPRESSION MAY EXPLAIN REDUCTION IN INFLAMMATION

R. McGregor[1,2], N. Chitalia[4], E. N. Lamperti[1], C.Hawrylowicz[3], D. Banerjee[4], G. Lombardi[1,2*], D. Goldsmith[3*], B. Afzali[1,2*]

1 Medical Research Council Centre for Transplantation, King's College London
2 National Institute for Health Research Biomedical Research Centre at Guy's and St Thomas' NHS Foundation Trust and King's College
3 King's College London School Of Medicine
4 Renal and Transplantation Unit, St Georges Healthcare NHS Trust
**Authors contributed equally to work*

wellcome trust Fellow

INTRODUCTION

- Chronic inflammation is a cardinal feature of chronic kidney disease (CKD), and plays a role in cardiovascular disease (CVD), which is the leading cause of death in patients with CKD
- Increased risk of CVD in CKD patients can not be explained by "traditional" CV risk factors alone.
- Recent focus has been on non-traditional risk factors: chronic inflammation and vitamin D (VitD) deficiency.

- Hypovitaminosis D is especially common in patients with CKD.
- VitD has anti-inflammatory properties
- We propose that one of the reversible mechanisms of CVD in CKD is chronic inflammation from hypovitaminosis D.
- We are carrying out a VitD repletion study, measuring CV markers before and after repletion with VitD or placebo in patients with early CKD and evidence of CVD.
- We hope to identify a link between chronic inflammation caused by VitD deficiency and accelerated CVD in CKD patients.

HYPOTHESIS AND AIMS

- The immunomodulatory activity of VitD repletion would result in improvement of chronic inflammation, which would improve cardiac function and mass, as well as myocardial composition.

METHODS

- 35 patients with hypovitaminosis D (serum 25 (OH)D levels between 12.5 to 75 nmol/L) and with CKD 3-4 and evidence of left ventricular hypertrophy were randomized to oral repletion with cholecalciferol or placebo for 52 weeks.
- Repletion regime was: observed oral Colecalciferol 100,000 administered to the trial participants at 0,4,8,12,24 and 42weeks.
- Blood cells isolated from these patients before and after repletion were tested ex vivo by immunophenotyping and memory responses to commonly encountered antigens and *in vivo* for responses to vaccination against Hepatitis B virus.

RESULTS

1. Flow cytometry assay design, validation and acceptable inter-assay variability

- We designed flow cytometry panels to immunophenptype the peripheral blood composition of each patient
- Flow cytometry panels where designed and validated using application settings and cytometer tracking and setup beads on FACS machine.
- This ensured laser intensities were normalised across all runs, enabling cytometer performance to be the same every day.

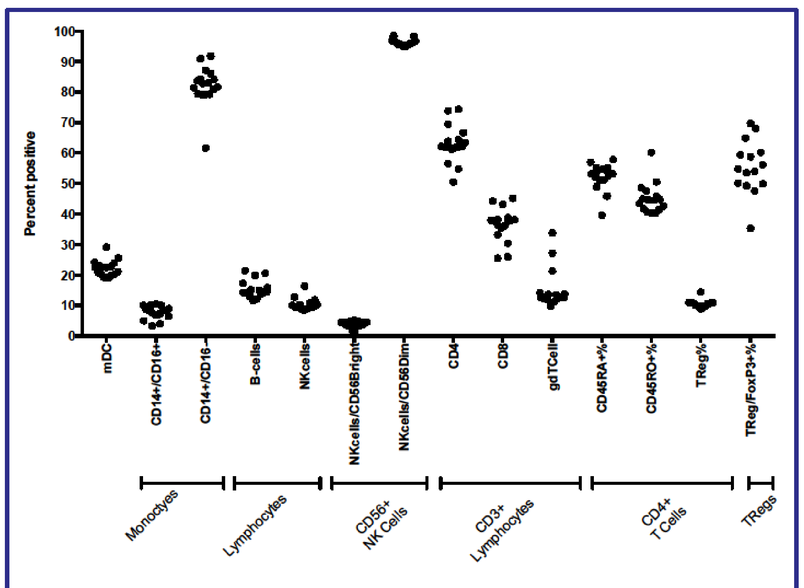

Figure 1| **Inter Assay Variability**| Multiple aliquots of PBMCs from a healthy control where was frozen down and stained with panels in parallel with patient samples. Thus values obtained from every run where used to asses variation between runs.

- Inter-assay variability was assessed by running of identical healthy control PBMCs with each phenotyping run 17 times.
- For most markers, there was minimal inter-assay variation between runs (Fig 1).
- Foxp3 showed the greatest variabilty between runs (Fig 1).
- Overall, the mean coefficient of variation was 15% across all antigens.

2. Immunophenotyping pre- and post-VitD repletion

- We immunophenotyped samples to determine peripheral blood cell composition of each patient at baseline and week 50 by flow cytometry (**Fig. 2A-B**).
- Most subjects showed some changes in immune cell composition.
- Analysis after un-blinding indicate a significant reduction in HLA-DR expression on mDCs and CD14+CD16+ monocytes (**Fig. 2C**).

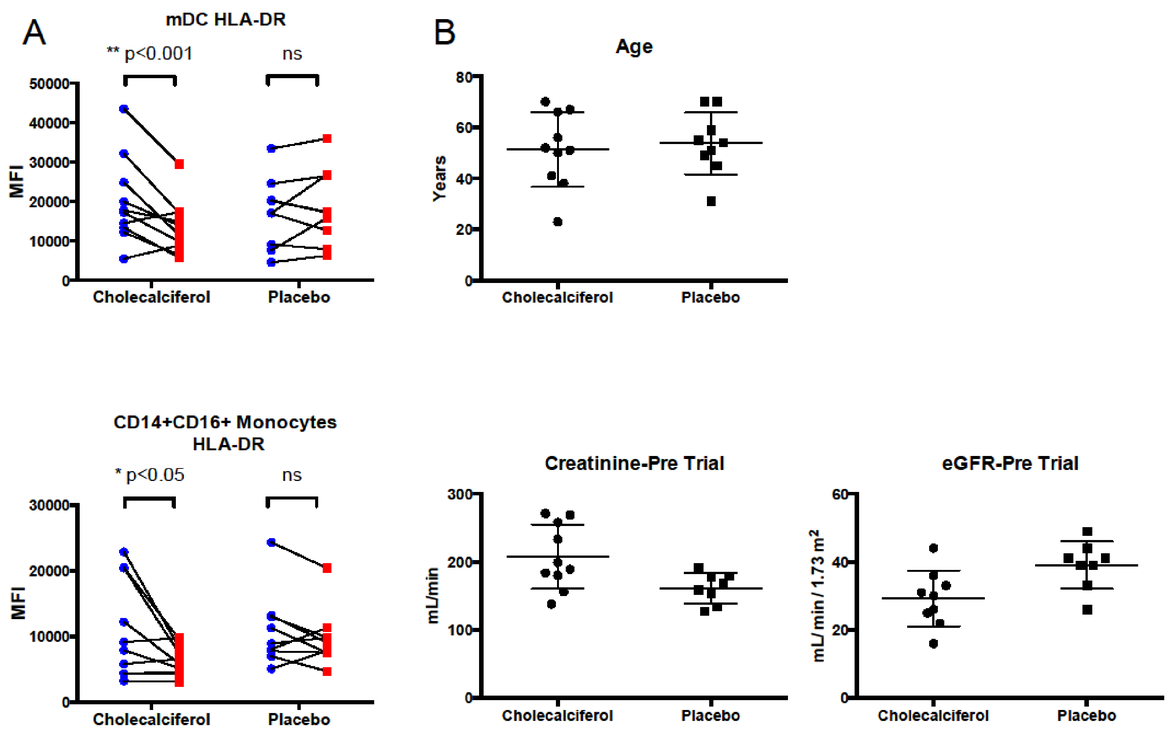

Figure 2| **Significant reduction in expression of maturation marker on mDCs and pro-inflammatory monocytes despite no significant differences in clinical parameters pre clinical trial** | A) Results following un-blinding indicate that functional/activation marker HLA-DR is down regulated following oral cholecalciferol repletion, on both mDCs as well as pro-inflammatory CD14+CD16+ monocytes (n=10 cholecalciferol, n=9 placebo) B) No significant difference in age or creatinine/eGFR pre clinical trial where observed.

3. Memory response assay pre- and post-VitD repletion

- PBMCs from patients were cultured with whole *C. albicans* preparation, a protein extract of *C. albicans* or a pool of 2 peptides from CMV (pp65 and gB). Anti-CD3/CD28 microbeads were used as a positive control and all measures were normalised to a medium alone condition.
- The majority of samples showed differences in both proliferation and cytokine production in the memory assay after 52 week trial period.

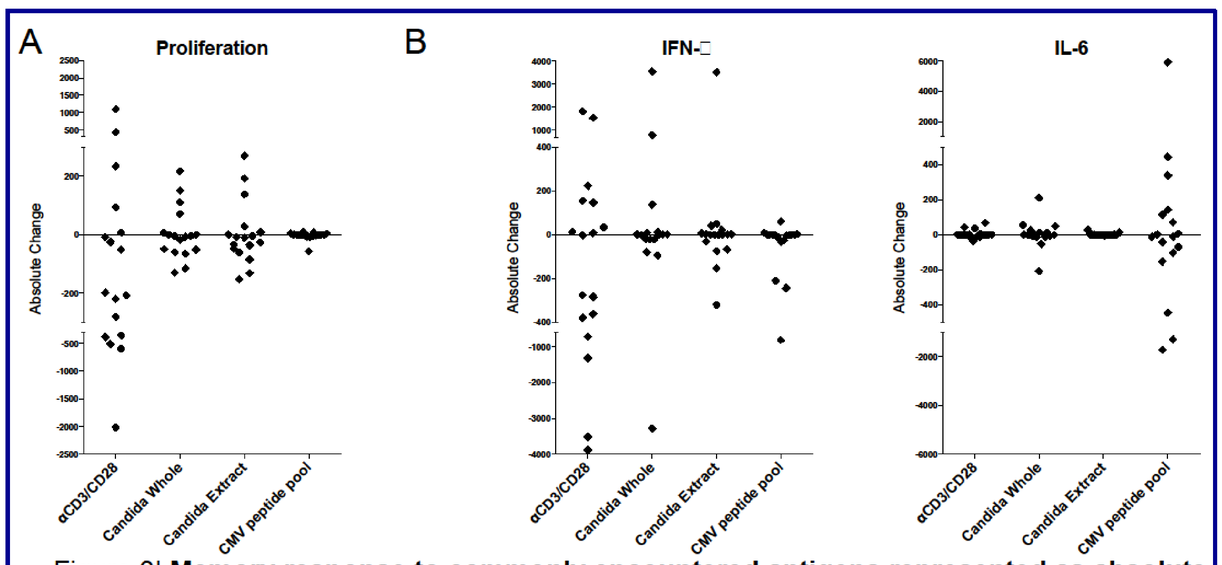

Figure 3| **Memory response to commonly encountered antigens represented as absolute change over 52 week period** | A) Proliferation by radioactive thymidine incorporation. B) Cytokine profile by CBA (cytometric bead array, similar to ELISA but using fluorescently labelled beads). All conditions normalised to medium alone condition and displayed as an increase (positive number) or decrease (negative number) in response after 52 weeks of oral cholecalciferol or placebo.

- 5 additional cytokines of relevance were also measured in response to common antigen exposure: IL-2, IL-4, IL-6, IL-17 and TNFα
- Further analysis is ongoing.

CONCLUSIONS/FUTURE WORK

- Flow cytometry panel and memory recall assay have been optimised and have been carried out on patient samples.
- Results show significant reduction of HLA-DR expression on mDCs and pro-inflammatory monocytes, post cholecalciferol repletion.
- This reduced expression of MHC-II on cells of the myeloid lineage indicates a reduced maturation and/or activation status of these cells, consistent with in-vitro studies of the effect of VitD and may indicate amelioration of pro-inflammatory status.
- Un-blinding of clinical parameters will allow correlation of cardiovascular indices with immunological parameters and levels of repletion will follow.

REFERENCES

- Betjes, M. G. H. (2013). Immune cell dysfunction and inflammation in end-stage renal disease. *Nature Reviews. Nephrology*, *9*(May), 255–265. doi:10.1038/nrneph.2013.44
- McGregor, R. *et al.* Vitamin D in renal transplantation - from biological mechanisms to clinical benefits. *Am. J. Transplant* **14**, 1259–70 (2014).
- Sterling, K. a, Eftekhari, P., Girndt, M., Kimmel, P. L., & Raj, D. S. (2012). The immunoregulatory function of vitamin D: implications in chronic kidney disease. *Nature Reviews. Nephrology*, *8*(7), 403–12. doi:10.1038/nrneph.2012.93

National Institute for Health Research

Guy's and St Thomas' NHS Foundation Trust and King's College London's Biomedical Research Centre
Working together to deliver better health through research

52 ERA 399--SP

J6) Chronic Kidney Disease. Nutrition, inflammation and oxidative stress.

Reuben McGregor

DOI: 10.3252/pso.eu.52era.2015

ePosters supported by F. Hoffmann- La Roche Ltd.

52nd ERA-EDTA CONGRESS London 2015 UNITED KINGDOM MAY 28 '15

PosterSessionOnline.com