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#### **Abstract Title: Evaluation *in vitro* of glucocorticoid sensitivity in transplant patients**

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**Body: Introduction and Aims** There is currently a lack of predictive methods to define glucocorticoid (GC) sensitivity, which is extremely variable among individuals. In particular, identifying patients in whom steroids will have immunosuppressive and toxic activities is important in transplantation. Therefore we have sought to establish an *in vitro* pharmacokinetic assay to investigate leukocyte proliferation and cytokine production, with the intention of applying such a test in transplantation.

**Methods** Peripheral blood mononuclear cells (PBMCs) were obtained from 21 healthy volunteers (n=21). These cells were stimulated to proliferate *in vitro* using PMA/ionomycin. Dexamethasone (Dex) was titrated into such cultures. Proliferation was measured by BrdU incorporation. Synthesis of cytokines (IL-2, IFN, IL-4 and TNF) was measured using a multiplex ELISA assay.

**Results** Individuals showed big differences in suppression of proliferation by Dex, and could be classified into GC sensitive, intermediate and resistant groups using IC<sub>50</sub> of 2x10<sup>-7</sup>M and 5x10<sup>-5</sup>M Dex, respectively. A strong correlation was found between the parameters I<sub>max</sub> and LogIC<sub>50</sub> (Pearsons R =-0.84, P=0.000). Validation experiments demonstrated excellent inter- and intra-assay reproducibility. The GC-sensitive individuals expressed significantly lower levels of IL-2, IL-4 and TNF than the insensitive ones (P<0.05), while there was no difference in the expression of IFN between GC sensitive and resistant groups.

**Discussion** Tests on PBMC proliferation and cytokine production *in vitro* have been used to investigate individual GC sensitivities. Here, the *in vitro* pharmacokinetics of Dex suppression in the proliferation assay divided the individuals according to Dex sensitivity and this was reflected by suppression of cytokine synthesis. Of note, IFN expression was not related neither to DEX dose nor to the production of other cytokines, perhaps because of the gene promoter lacks an NF-B motif. Analysis of GC sensitivity in *in vitro* assay prior to initiation of steroid therapy may help clinicians make a rational choice of immunosuppressant agent and appropriate dose, thereby limiting the unnecessary exposure of patients to an agent with numerous side effects.