Pump-Priming Grant

Report

Miss Lente Lerink

Title: Cell death pathways activation in donor kidneys and associations with transplant outcomes

Objective

The objective of the study was to examine whether (specific) cell death pathways are activated in donor kidneys prior to transplantation, and if this is related to transplant outcomes. Four different donor groups were included: DBD and DCD donors with good and poor 12-month transplant outcomes.

Method

Paraffin-embedded (FFPE) biopsies were requested via the QUOD biobank initiative to spatially and semi-quantitatively detect cell death activation. Four groups of 15 donors each were included in this study: DBD and DCD donors with good or poor transplant outcomes (12-month eGFR ≥60 ml/min/1.73 m2 or ≤30 ml/min/1.73 m2, respectively).

Based on a review of the recent literature on cell death, four cell death markers were identified that are suitable for detection of four separate cell death pathways using immunofluorescence: cleaved caspase 3 for detection of apoptosis, phosphorylated MLKL (pMLKL) for detection of necroptosis, cleaved GSDMD for detection of pyroptosis, and translocated HMGB1 for detection of cell death in general.

After extensive optimization of a multiparameter staining technique using human rejected kidney and discarded kidney biopsies, slides of the FFPE biopsies were stained using selected antibodies and the Opal multiplex system and the Vectra spectral imaging platform. This system facilitates the simultaneous detection (and semi-quantification) of the activation of multiple cell death pathways in one slide using immunofluorescence.

Results

Multiple cells from the different kidney donor groups showed ample activation of cell-death pathways in the proximal tubili and co-expression of cell death activation markers. There was no difference in the percentage of positive (i.e., dying) cells, nor in the activation of specific cell death pathways, between the different kidney donor groups; no correlations with donor type or 12-month transplant outcome were found.

Outputs (publications/presentations)

Data analysis just finalized, manuscript in preparation.

Next Steps (what is it leading to):

We were able to develop a multiparameter staining platform that allows for quantification of key cell death pathways. Activation was mainly observed in the tubulus epithelium and the proportion of

positive cells was high. The latter phenomenon may reflect the timing of the biopsy (pre-transplantation biopsies).

Our findings facilitate a deeper understanding of the activation of cell death pathways in donor kidneys, thereby aiding the development of therapeutic interventions. As research into the inhibition of cell death in donor kidneys using specific inhibitors is ongoing, our finding that multiple cell death pathways can be simultaneously activated is very relevant; inhibiting one pathway may not be enough to reduce the negative effects of cell death or may even cause an undesired imbalance, which could mean that multiple pathways need to be targeted in parallel. Still, as our results did not show a significant difference between the kidney donor groups and outcomes, the overall effect of cell death activation on 12-month outcomes may be limited, and the potential of therapeutic intervention could therefore also be limited.