Published in final edited form as:

Transplantation. 2019 November ; 103(11): 2217–2218. doi:10.1097/TP.0000000000002597.

Circulating Donor Mitochondrial DNA: Tales the Dead May Tell

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The need for kidney transplantation continues to outpace the number of available kidneys, necessitating efforts to maximize the use of all viable organs. Several strategies are variably ongoing to increase organ supply including increasing donor enrollment by creating public awareness regarding organ donation, optimizing organ retrieval and allocation, technologies to improve viability of obtained organs with prolonged cold ischemia time, and increasing organs donated after circulatory death (DCD). The number of organs discarded after procurement remains near 20%, due to both the widening donor pool (ie, use of older donors with greater comorbidities) and due to unquantifiable factors such as regional variations and surgeon preference.[1] Organs procured from DCD donors are twice more likely to be discarded than organs from standard criteria donors.[1] Interestingly, neither intuitive clinical metrics like duration of agonal phase[2] nor well-calibrated hemodynamic metrics[3] universally correlate well with long-term allograft function in DCD transplants. Similarly, neither premortem donor acute kidney injury[4] nor preperfusion procurement biopsies correlate with allograft outcomes.[5] On the other hand, kidneys transplanted, where mate kidneys were discarded, showed acceptable long-term outcomes, suggesting that potential donor organs are still being discarded after procurement.[5] Crucially, long-term allograft failure requiring retransplantation remains a challenge adding on to the kidney waitlist and increasing demand on the supply of organs. Hence, biomarkers that help optimize organ selection, that is, improve selection of organs for either discard or transplantation, that could subsequently translate into improved allograft function of organs that are transplanted, are an ongoing need.[4]

In this issue of the journal, Han et al[6] report data from a retrospective multicenter Chinese cohort linking levels of a novel plasma biomarker—mitochondrial DNA (mtDNA) from DCD donors to immediate and intermediate-term allograft outcomes in the recipient.[6] Human mtDNA is ≈17 000 bp in length, encoding 37 genes, that is, 22 tRNAs, 2 ribosomal RNAs, and 13 polypeptides, and is the only non-nuclear source of DNA (reviewed in Hu et al[7]). Mitochondria are the site of oxidative phosphorylation and the primary source of cellular reactive oxygen species. mtDNA has no histones and are organized as circular, covalently closed, double-stranded DNA molecules. The higher reactive oxygen species exposure coupled with lack of protective nucleosomal structure make mtDNA uniquely susceptible to ischemia-reperfusion (I/R) injury (eg, during transplantation). MtDNA

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The authors declare no funding or conflicts of interest.

fragments are released upon injury both as a consequence of cell death and via specialized channels in live cells after injury. The latter pathway also signifies the tight regulation of mtDNA release in homeostasis.[8]

In the current study, the authors hypothesized that, in view of prior data correlating mtDNA to outcomes in coronary disease and sepsis,[7] and due to its unique role in I/R injury, mtDNA levels obtained from DCD donors before organ procurement would correlate with donor organ injury and, in turn, with allograft outcomes in recipient. To minimize impact of recipient factors on these outcomes, they included only mate kidneys from same donors that also sustained similar rates of immediate graft dysfunction, that is, delayed graft function (DGF)/slow graft function (SGF)/primary non function (PNF)/Discard (75 donors/141 kidneys—Discovery cohort, and 16 donors/28 kidneys— validation cohort). MtDNA was assayed by quantitative polymerase chain reaction after isolating plasma cell-free DNA and reported as a ratio of mitochondrial gene *ND1* amplicons-to-human single copy gene *36B4* amplicons, with post-test batch normalization. The authors initially observed that donor- or d-mtDNA levels correlated with premortem acute kidney injury (by creatinine) and biopsy staining for kidney injury molecule-1. On the basis of these data, they derived a cutoff value and showed that d-mtDNA levels >0.114 associated with graft injury in the recipient denoted by DGF (68% versus 16%, $P < 0.001$). Further in the discovery cohort, levels >0.243 were associated with 100% DGF and 44% PNF rates. Notably, reported rates of PNF are near 1% for all transplants,[1] and 4% to 7% in DCD kidneys.[2] A significant ordinal relationship was seen with increasing levels of immediate graft dysfunction and mean d-mtDNA levels. The authors then defined and adopted a single graft dysfunction end point, that is, reduced graft function (RGF). The diagnostic performance of d-mtDNA for predicting RGF was superior to donor terminal creatinine, warm ischemia, and kidney donor profile index. An additive multivariable model including all significant predictors provided an area under the curve of 0.844 for RGF. These results for RGF were then validated in the smaller validation cohort with high sensitivity and specificity. Most importantly, in uni- and multivariable analysis, d-mtDNA levels significantly associated with 6-month allograft function and 12-month graft survival.

While these data report a novel biomarker for detecting severe renal injury and PNF in DCD kidneys, the small size of the validation cohort, the single mtDNA gene used to identify mitochondrial injury and DNA release, the semiquantitative nature and variability of quantitative polymerase chain reaction all relay the need for validation of both technique and biomarker in larger cohorts. Notably, the identified association of mtDNA with graft survival was also not independent of DGF itself. Because mtDNA release is generic to I/R injury in multiple organs, the renal source of the elevated mtDNA is not confirmed in these data. Analogously, the impact of nonrenal organs utilized for transplantation in donors with high d-mtDNA levels needs to be separately examined.

Nonetheless, these data could have important mechanistic implications. The regulated release pathways of mtDNA with cell injury likely exist because free mtDNA acts as a danger-associated molecular pattern signal binding pattern recognition receptors especially TLR9, promoting proinflammatory cytokines (interleukin-6 and tumor necrosis factor-α) and aggravating I/R injury.[9] In the context of transplantation, activation of innate immune

cells via TLR signaling promotes the migration of mature antigen-presenting cells to secondary lymphoid tissues where they trigger primary T-cell and B-cell responses, thus recruiting the adaptive immune system and culminating in an alloresponse.[10] Hence the associations seen with longer-term outcomes likely have true mechanistic basis. These data also raise the possibility of specifically testing TLR-signaling inhibitors in the context of elevated mtDNA levels in DCD or standard criteria donors. From the standpoint of clinical application for organ allocation, speculatively, a combined model using mtDNA and other biomarkers such as YKL40, along with well-known clinicopathologic factors could further improve organ risk stratification, moving the ball forward in the quest for improved long-term graft survival.

ACKNOWLEDGMENTS

M.C.M. acknowledges research support from American Heart Association Scientific development Grant, 15SDG25870018, and from the National Institutes of Health National Institute of Allergy and Infectious Diseases via a Pilot study of the Clinical Trials in Organ Transplantation-19-2U01AI063594-11.

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