

# Analysis of donor-derived cell-free DNA with 3 year outcomes in heart transplant recipients

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**Purpose:** Elevated donor-derived cell-free DNA (dd-cfDNA) has been observed in transplant patients undergoing acute rejection, but the relationship between dd-cfDNA and long term outcomes has not previously been studied. In this exploratory study, serially measured dd-cfDNA from heart transplant patients is used for predict 3-year patient adverse outcomes of death, retransplantation, hemodynamic compromise or graft dysfunction.

**Methods:** cfDNA was extracted from 166 plasma samples representing 48 patients from the CARGO II observational study and dd-cfDNA was quantified using a targeted NGS assay. For each patient, the median and maximum of serial dd-cfDNA scores were calculated. The summarized scores for patients with long-term adverse outcomes and patients without adverse outcomes were compared via two-sample testing and AUC-ROC analysis. Within-patient variability of dd-cfDNA scores for patients who had at least 4 scores was also computed (17 patients total).

**Results:** Eight of the 48 patients (40 samples) had a adverse outcome and serial dd-cfDNA measurements. Patients were  $49 \pm 13$  years old, 79% male and 96% caucasian, with no statistically significant difference between groups. Within-patient median dd-cfDNA was significantly correlated with outcome ( $p = 0.02$ , AUC 77%, 95% CI 59%-92%) but within-patient maximum was not ( $p = 0.11$ , AUC 68%). Analysis using all dd-cfDNA results from every patient was also not significantly correlated with outcomes,  $p = 0.09$ . The AUC for predicting long term outcomes in the CARGO II dataset using variability of gene expression profiling scores was 72%, dd-cfDNA variability was noticeably increased in four patients who an adverse outcome and had four serial AlloSure scores when compared to 13 patients who did not have an adverse outcome.

**Conclusion:** Median dd-cfDNA score is correlated to long-term outcome. This result suggests that dd-cfDNA merits further investigation as a prognostic marker of long-term risk in heart transplant patients.