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Association of two non-invasive biomarkers with 10-year All Cause Graft Loss in a Multicenter Prospective Cohort

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I have no financial disclosures

Purpose

- Lower first-year rejection levels have only slightly improved long-term graft survival, kidney transplant (KTx) is still the preferred therapy for ESKD.
- A major contributor to long-term graft loss is alloimmune rejection, particularly subclinical acute rejection (SubAR) and donor-specific antibody (DSA) development.
- Findings from protocol biopsies have demonstrated that even subclinical rejection greatly reduces allograft survival.
- Noninvasive gene expression profiling (GEP) and donor derived cellfree DNA (ddcfDNA) may identify SubAR and are linked to poorer clinical outcomes, as shown by the CTOT08 multicenter U.S. research.
- Though GEP and ddcfDNA can detect SubAR early, their predictive value for ten-year all-cause graft loss (ACGL) has not been totally studied—therefore this study's goal is to investigate the prognostic power of these biomarekrs.

Methods

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- The CTOT-08 was a multicenter, 24-month observational study that was designed to develop noninvasive biomarkers predictive of subclinical rejection and 2-year allograft outcomes.
- Subjects were matched with the SRTR database to collect 10-year graft and patient survival.
- The aim of this analysis is to evaluate the relationship between GEP (TruGraf) & dd-cfDNA (TRAC)) performed serially for 2 years and 10-year all-cause graft loss (ACGL).
- A Kaplan Meier Curve evaluated event-free survival between amongst the population based on the number of positive TruGraf GEP or TRAC dd-cfDNA over the 24-month prospective follow-up.
- A Cox Regression time-to-event analysis was planned using baseline covariates (Black race, diabetes as cause of ESKD, living donor, recipient age, donor age) as well as clinical acute rejection events and positive GEP as time-varying covariates.

Methods

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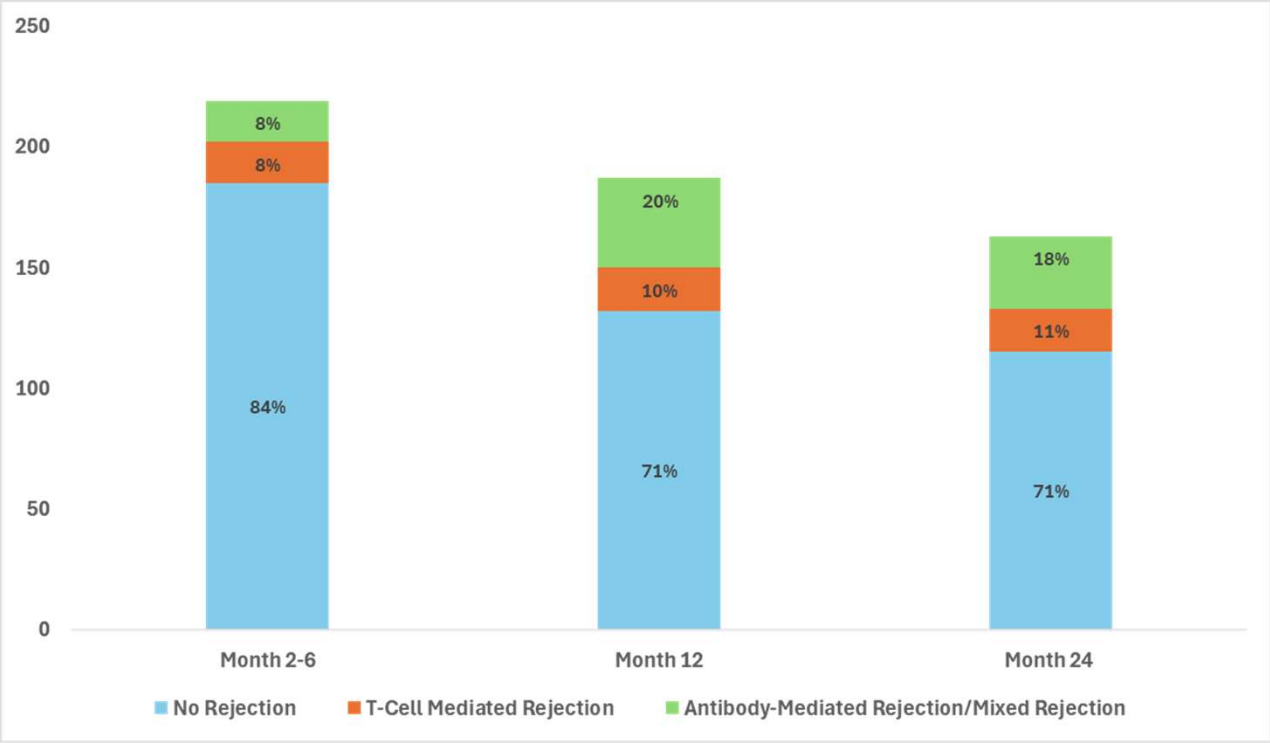
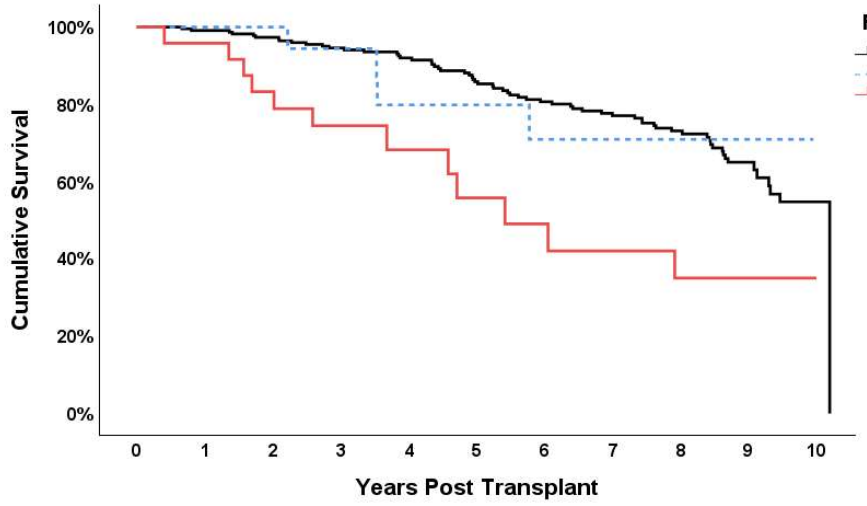
- Biomarker Definitions
  - GEP Positive: Score >50
  - dd-cfDNA Positive: >0.69%
- Biomarker quartiles created based on frequency of positive results (for GEP and dd-cfDNA)
  - (Quartile 1: 0 positive tests, Quartile 2: 1-2 positive tests, Quartile 3: 2-4 positive tests, Quartile 4: 5 or more positive tests)
- ACGL defined as death with functioning graft or graft failure
- Demographics
  - Median age: 52 years
  - 65% male; 64% White; 20% Black
  - 55% living donor transplants
  - All on tacrolimus; most on mycophenolate ± steroids
  - Similar eGFR at 2 years regardless of later graft loss

Surveillance Biopsy Results – CTOT 08



- Proportion of patients experiencing rejection rose from 16% at months 2–6 to 29% by month 12 and stayed similar at month 24.
- ABMR/mixed rejection increased significantly from 8% (months 2–6) to 20% (month 12), indicating that humoral immune responses become more prominent.
- TCMR increased slightly over time but remained low across all periods.
- Despite rising ABMR/TCMR, 71% of patients remained rejection-free at 12 and 24 months.

Patient and Allograft Survival by Type of Early Subclinical Rejection



No Rejection	228	226	216	201	169	151	137	123	93	48	5
T-cell Mediated	22	21	19	17	9	8	7	7	5	3	0
Antibody Mediated	24	23	19	15	11	9	7	6	5	1	0

- ABMR is strongly associated with early and progressive graft loss, underscoring the need for close monitoring and targeted interventions.
- TCMR, particularly if treated promptly, may not significantly impact long-term survival in this cohort.
- Surveillance biopsies provide early insight into immune injury, and non-invasive biomarkers could help identify these risk patterns without requiring invasive procedures.

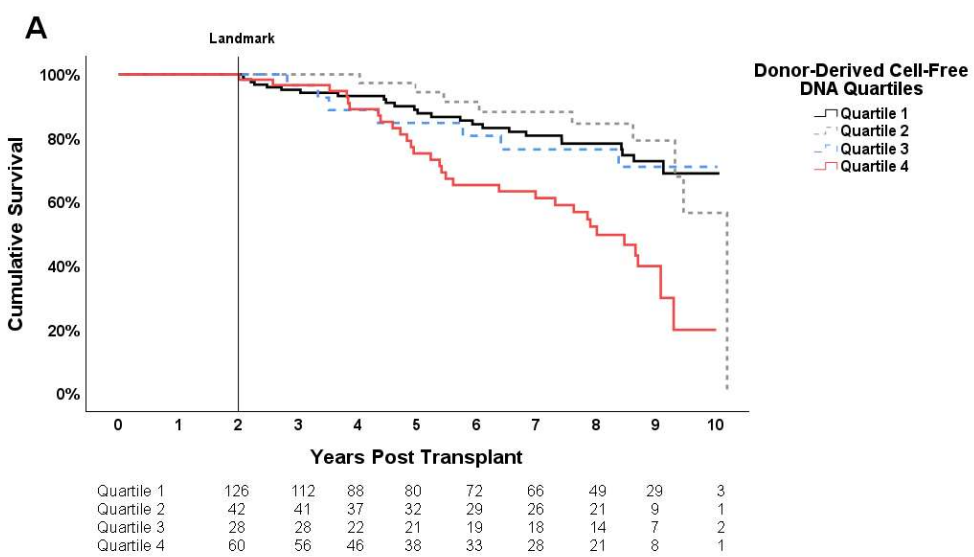
Results

The final landmarked Cox model included:

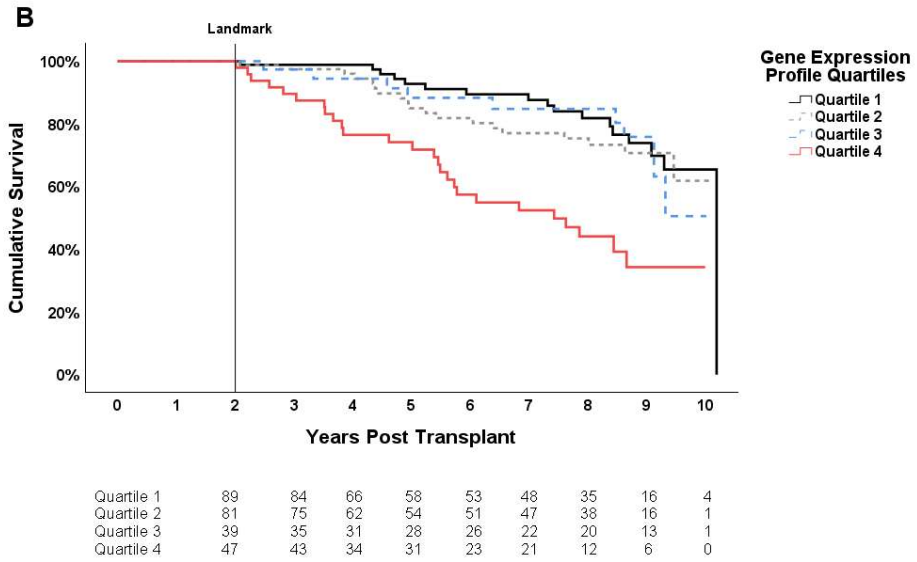
- Clinical acute rejection (aHR 2.58 (1,30,5.13) for 1 rejection, aHR 3.41 (1.07,10.85) for 2 rejections)
- Diabetes (aHR 1.71 (0.99,2.96))
- Recipient age (aHR 1.02 (1.00,1.04))
- The highest quartile of positive GEP (aHR 2.44 (1.45,4.10))
- The highest quartile of positive dd-cfDNA (aHR 2.91 (1.77,4.78)).

In the time-varying model, having both biomarkers positive at the same time carried the greatest association with ACGL (aHR 4.29 (2.10,8.76)). Model C-index: 0.78

Patient and Allograft Survival Between Biomarker Population Quartiles



dd-cfDNA



GEP

Discussion

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Findings

- Gene Expression Profiling (GEP) and donor-derived cell-free DNA (dd-cfDNA) are each independently associated with long-term graft loss showing complementary predictive value when used in tandem.
- Noninvasive biomarkers outperform subclinical acute rejection (SubAR) histology in predicting long-term outcomes.

Limitations

- Post-hoc analysis: results are associative, not causal. No follow-up data on clinical events occurring between years 2–10 post-transplant.
- Lacks data on de novo donor-specific antibody (DSA) development, center specific IS therapies, which could influence interpretation of rejection mechanisms.

Strengths

- Multicenter, prospective study design.
- Long-term linkage to outcomes, supporting real-world applicability of biomarker findings.

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Thank you