Circulating Donor-Derived Cell-Free DNA: A Novel Non-Invasive Biomarker for Immune Surveillance in Vascularized Composite Allotransplantation

Presenter: William J Rifkin, MD

Co-Authors: Adam Jacoby, MD, Hansjorg Wyss Department of Plastic Surgery, New York University Langone Health, New York, NY; Piul S Rabbani, PhD, Hansjorg Wyss Department of Plastic Surgery, New York University Langone Health, New York, NY; Bruce E. Gelb, MD, Hansjorg Wyss Department of Plastic Surgery, New York University Langone Health, new york, NY; Daniel J. Ceradini, MD, Hansjorg Wyss Department of Plastic Surgery, New York University Langone Health, New York, NY; Eduardo D Rodriguez, MD, DDS, Hansjorg Wyss Department of Plastic Surgery, NYU Langone Health, New York, NY

Affiliation: Hansjorg Wyss Department of Plastic Surgery, New York University Langone Health, New York, NY

BACKGROUND

Current diagnosis of rejection in vascularized composite allotransplantation (VCA) relies on correlation of clinical examination with skin histopathology. However, these methods remain both subjective and semi-quantitative, and frequent biopsies carry the risk of infection, scarring, and/or inciting rejection through immune activation. There remains a need for novel, noninvasive methods of diagnosing rejection in VCA. Cell-free DNA (cfDNA) refers to circulating fragments of DNA in the blood originating from cells undergoing cell injury and death. Currently, cfDNA is an established biomarker in both prenatal testing and oncologic screening/prognostics. Based on promising results in solid organ transplantation, here we evaluated whether circulating donor-derived cfDNA (dd-cfDNA) could be used as a noninvasive biomarker to detect rejection in VCA.

METHODS

One face/upper extremity and two face transplant recipients were followed longitudinally, and plasma samples were collected prospectively at all follow-up appointments in combination with clinical photography and allograft biopsies. Peripheral blood samples were collected and plasma levels of dd-cfDNA measured via targeted amplification and sequencing of a validated panel of single-nucleotide polymorphisms (SNPs). The percentage of dd-cfDNA to total cfDNA (dd-cfDNA%) was calculated for each sample and correlated with rejection status based on clinical exam and histopathology.

RESULTS

Recipient 1 went demonstrated undetectable levels of dd-cfDNA pre-transplantation, a peak of 1.1% on postoperative day (POD) 26, and a subsequent drop to undetectable levels, all in the absence of rejection episodes. Recipient 2 experienced an episode of biopsy-proven acute rejection on postoperative day (POD) 537, which corresponded with an elevated level of dd-cfDNA (1.3%). After treatment with pulse steroids and taper, acute rejection resolved both clinically and histologically, which correlated with return to baseline levels of dd-cfDNA, and

remained below 1% in the absence of rejection (0.44-0.86% dd-cfDNA). Recipient 2 experienced an acute rejection episode on POD 1466, which also correlated with an elevated dd-cfDNA level (4.4%). After treatment with pulse steroids, rejection resolved clinically and histologically, with a subsequent drop in dd-cfDNA (1.2%).

CONCLUSION

These results suggest that elevated levels of dd-cfDNA, representing allograft injury, appear to correlate with acute rejection of the allograft. While further research and a larger sample are needed to confirm its validity, this safe, simple, and noninvasive test may be used for rejection surveillance, enabling more frequent and quantitative assessment while also complementing traditional clinical and histopathological data for decision-making.