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Technology-driven Performance Improvement of a Biomarker Panel in Kidney Transplant: OmniGraf

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Abstract

Here we present the impact of the transition from TruGraf microarray to TruGraf PCR on the performance of OmniGraf, the combination of TruGraf Gene expression and TRAC dd-cfDNA tests. Using the same biopsy-paired samples as previously published, we saw an improvement in the NPV from 88% to 94% when both assays were negative. Perhaps more importantly, we observed an increase in the PPV from 81% to 89% when both tests were positive. False negative results were reduced from 31% to 17%, while true negative results improved from 74% to 81%. Within our cohorts, we observed 26.2% of results to be positive for one test and negative for the other: 11.7% showed elevated TRAC (+) and a negative TruGraf (-); 14.5% showed a TruGraf positive (+) and low TRAC score (-). The previous publication demonstrated that TruGraf microarray was significantly better at detecting subclinical TCMR and TRAC was significantly better at detecting subclinical ABMR, highlighting the importance of the combination of the tests. The methodological improvement in TruGraf technology increased its detection of both TCMR and ABMR subtypes of rejection, leading to a higher NPV and PPV. In the field of transplant biomarkers, where high NPV values have been the focus, we present novel clinical validation data on the first commercial biomarker panel with a high PPV. With the data presented here, OmniGraf results provide a high probability of either immune quiescence or subclinical rejection to support clinical decision-making.

Keywords: Subclinical rejection • Gene expression profile • Dd-cfDNA • Biomarker • Kidney transplant

Abbreviations: ABMR: Antibody Mediated Rejection; CMS: Centers for Medicare & Medicaid Services; CTOT-08: Clinical Trials in Transplantation 08; Dd-cfDNA: Donor-Derived Cell-Free DNA; GEP: Gene Expression Profile; NPV: Negative Predictive Value; PPV: Positive Predictive Value; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; SubAR: Subclinical Rejection and Borderline Inflammation; TCMR: T-Cell Mediated Rejection

Letter

Within the novel and evolving commercial transplant biomarker era, it has become apparent that "integrating…two mechanistically distinct biomarker assays could allow for the better detection of subclinical acute rejection in kidney transplant recipients" than either individual biomarker [1]. Analyses have demonstrated both consistent prevalence and incidence rates of subclinical rejection and borderline inflammation (subAR), as well as adverse outcomes of graft injury and reduced allograft survival [2-4]. Despite the need to identify and treat subAR, the inability to choose an ideal timepoint to reveal subAR along with the cost and inconveniences of surveillance biopsies have led to inefficient protocols to address this important pathology. The TruGraf® Gene Expression Profile (GEP) assay is the only commercially available biomarker specifically targeting subAR. In an attempt to improve the Positive Predictive Value (PPV) for subAR, Park and the CTOT-08 study group published the performance characteristics of combined use of TruGraf and TRACTM donor-derived cell-free DNA (Dd-cfDNA) associated with modernized biopsy readings from a subset of the original CTOT-08 publication [5]. TruGraf results of "TX" are considered negative and "not-TX" are considered positive while TRAC results of <0.7% are considered negative and results of ≥ 0.7% are considered positive. The investigators found that combining these distinct biomarkers improved the Negative Predictive Value (NPV) from 82% and 84%, individually, to 88% when both tests were negative. Even more importantly, the PPV increased from 47 and 56%, individually, to 81% when both tests were positive [5].

While the technology behind TRAC Dd-cfDNA is relatively static, we saw an opportunity to improve upon diagnostic capabilities of the TruGraf assay, while simultaneously improving upon its operational ease and clinical performance. Development of the TruGraf assay was originally performed via a high-throughput analysis of gene expression on the Affymetrix microarray system (ThermoFisher Scientific, Waltham, MA). Microarray, while useful as a discovery tool to screen thousands of genes in a single experiment, is a source of high assay variability and can have results influenced by each step of the complex hybridization assay. It is also time-consuming, labor-intensive and requires large amounts of pure RNA. This leads to a turnaround time of potentially 7 days or more.

Using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and microfluidics on the Fluidigm Biomark HDTM system (Fluidigm, South San Francisco, CA) provides the potential for more rapid and quantitative analysis of gene expression, while requiring less RNA input and substantially reducing turnaround time. We performed analytical and clinical validation between the RT-PCR and microarray processes; not only did we find them to be analytically non-inferior, but we observed strong improvement in the clinical performance

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(in press). The Palmetto GBA Molecular Diagnostic Program (MolDX®) agreed and approved conversion of TruGraf methodology from microarray to PCR for CMS coverage. Further, we found that we could reliably provide sufficient sample for both TRAC Dd-cfDNA and TruGraf GEP using one single HemaSure-OMICS tube (Mawi, Hayward, CA) containing 6 mL of blood, a significant improvement from the ~15 mL of blood previously required to run both. The resulting combination biomarker panel is OmniGrafTM.

Here we present the impact of the transition from TruGraf microarray to TruGraf PCR [5]. Using the same biopsy-paired samples as Park, we saw an improvement in the NPV from 88% to 94% when both assays were negative. Perhaps more importantly, we observed an increase in the PPV from 81% to 89% when both tests were positive (Figure 1A and 1B). False negative results were reduced from 31% to 17%, while true negative results improved from 74% to 81%. With OmniGraf, we observed 26.2% of results to be positive for one test and negative for the other: 11.7% showed elevated TRAC (+) and a TX TruGraf (-); 14.5% showed a TruGraf not-TX (+) and low TRAC score (-). Park and colleagues commented that TruGraf was significantly better at detecting subclinical TCMR and TRAC was significantly better at detecting subclinical ABMR; however, this methodological improvement in TruGraf technology increased its detection of all subtypes of rejection (Figure 1C and 1D). When considering results that do not agree, it is important to note that each test preferentially detects a different type of rejection (TruGraf ACR and TRAC AMR).

In the field of transplant biomarkers where high NPV values have been the focus we present novel clinical validation data on the first commercial biomarker panel with a high PPV. With the data presented here, OmniGraf results provide a high probability of either immune quiescence or subclinical rejection to support clinical decision-making.

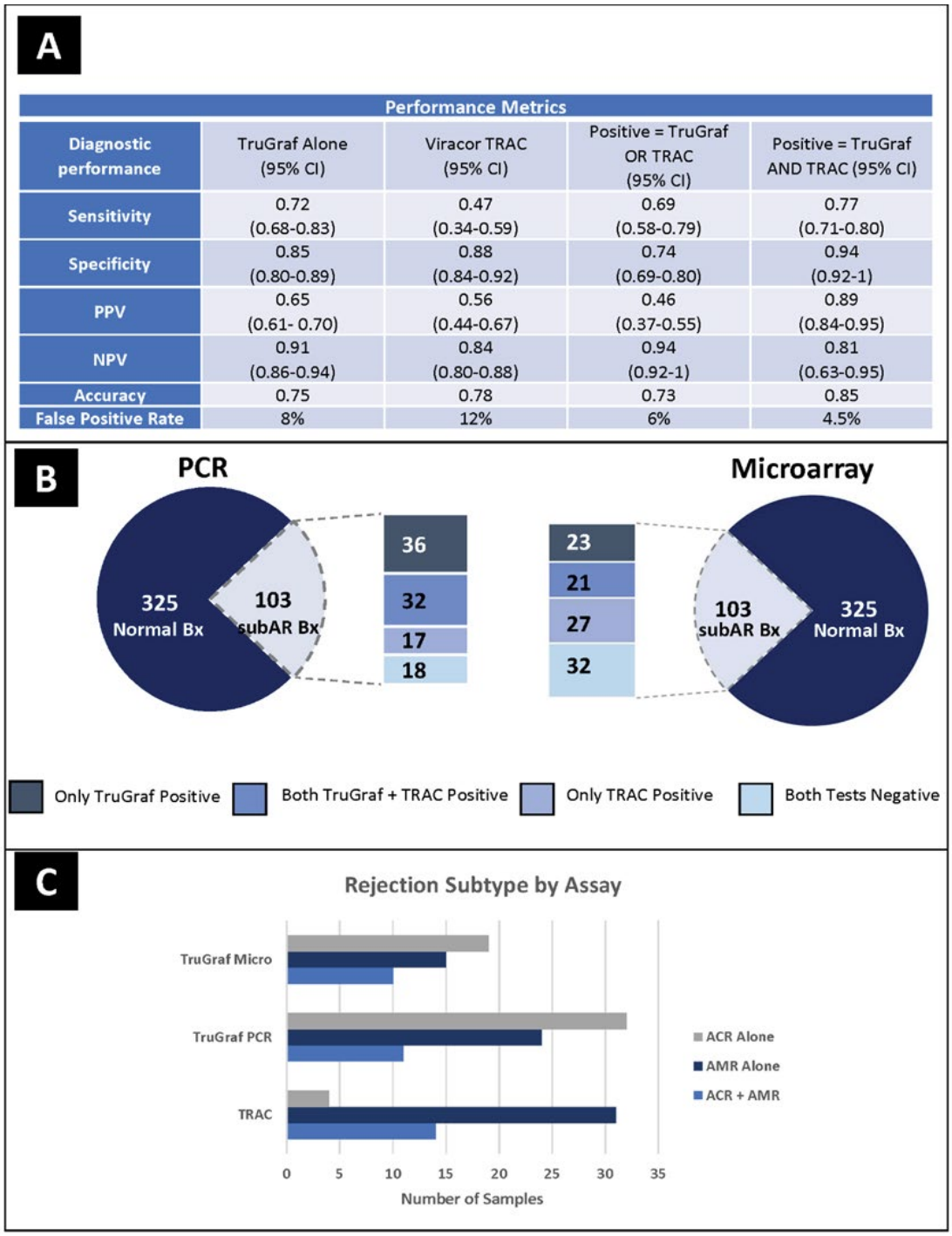

Figure 1. A) Performance metrics of PCR, B) comparison of PCR and microarray sample data and C) comparison of the rejection subtypes by assay type.

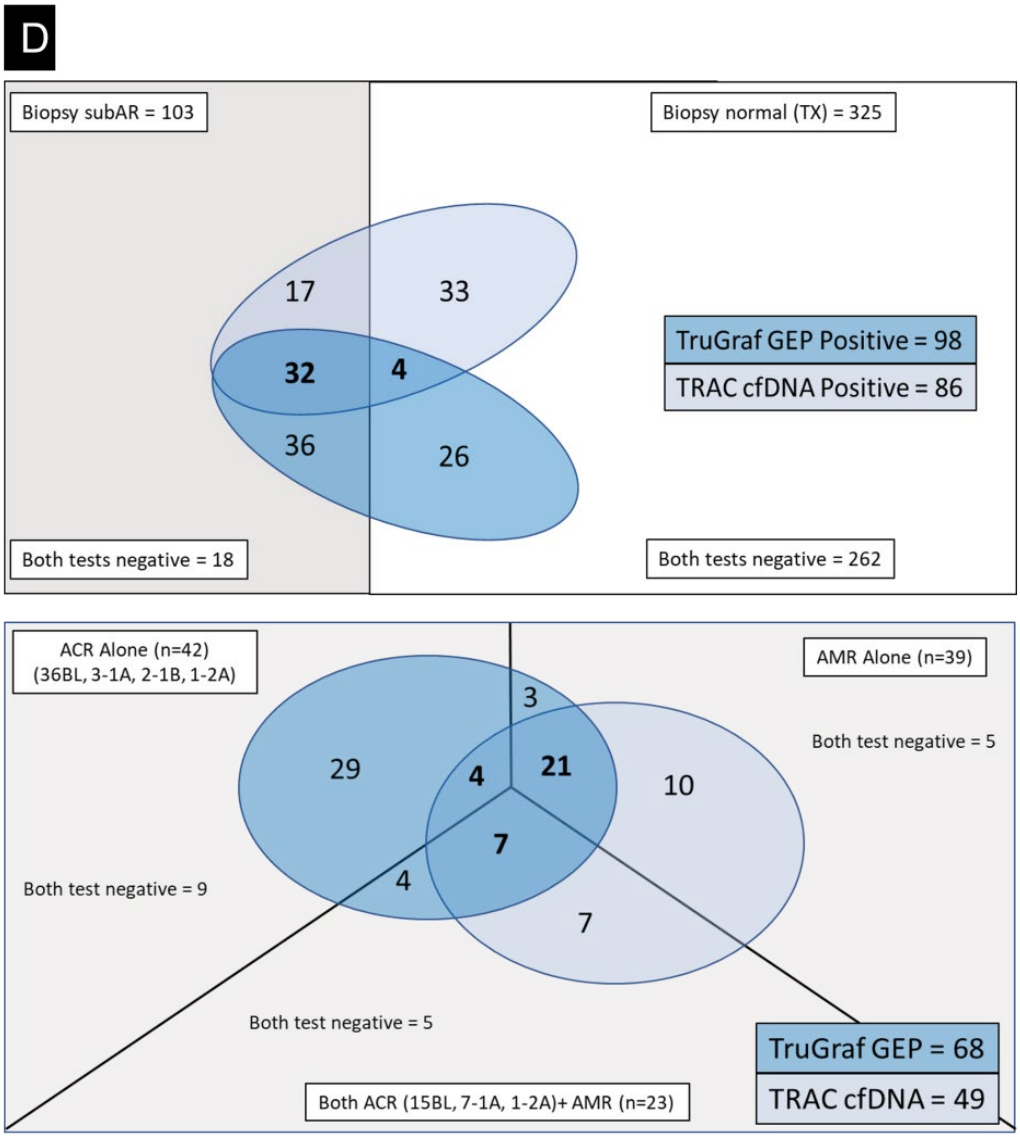

Figure 1. D) Overlap of subclinical rejection/no rejection samples by assay (upper); breakdown of subclinical rejection samples by type of rejection (lower) according to Banff 2019 criteria.

Acknowledgement

None.

Conflict of Interest

The author shows no conflict of interest towards this manuscript.

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