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# Variability by Donor Type, Sex, Race, BMI and Within Practitioner in Viscoelastic Response (VisR) Ultrasound Derived Mechanical Anisotropy Assessments in Healthy Kidney Allografts, *In Vivo*

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*Abstract*— **Degree of mechanical anisotropy in renal cortex can be assessed by evaluating the ratio of viscoelastic response (VisR) parameters achieved when the long axis of a spatially asymmetric ARF excitation PSF is aligned along versus across nephrons. In this work, intra-observer reproducibility and variability in VisR derived DoA assessment in renal cortex due to donor type, sex, race, and BMI is evaluated in healthy human kidney allografts, *in vivo*. Transcutaneous, i*n vivo* VisR imaging was performed in 20 kidney allografts 2 months post-transplantation using a Siemens Acuson Antares™ imaging system and a VF7-3 linear array transducer. Ratios of VisR derived relative elasticity (RE), relative viscosity (RV), and peak displacement (PD) obtained with the ARF short-axis aligned along versus across nephrons were calculated. Patients were divided based on their sex (male: N=14 versus female: N=6), race (African American: N = 6 versus Caucasian: N=14), donor type (living: N=14 versus deceased: N =6), and BMI (BMI >30: N= 8 versus BMI< 30: N= 12). Among the 20 patients, 16 patients were also imaged one month later (i.e., 3 months' post-transplantation) to study intra-operator variability. All patients had stable serum creatinine levels without clinical indication for biopsy. PD, RE, and RV ratios were not statistically different (Wilcoxon, p>0.05) due to sex, race, donor types, or BMI. The median absolute percent difference in (PD, RE, RV) ratios were (23, 13, 23) due to sex, (5, 3, 3) due to race, (0.35, 14, 13) due to donor type, and 8.6, 6, 12) due to BMI. Ratios of PD, RE, and RV were not statistically different between 1st and 2nd imaging events with mean percent difference in (PD, RE, and RV) ratios of (-0.04, -0.11, and - 0.01). These findings suggest that VisR outcome measures are robust to potentially confounding demographic factors and reproducible by a trained practitioner.**

**Keywords— Viscoelastic Response (VisR) ultrasound, Kidney Transplant; Chronic Kidney Disease; Anisotropy; ARFI; Shear Wave Elasticity Imaging; Ultrasound.**

## I. Introduction

The most cost-effective and favorable treatment for patients who are suffering from chronic kidney disease is renal transplantation [1]. The primary objective is to provide the transplant recipient with sufficient renal function to avoid dialysis, but the majority of allografts do not function for the remainder of a recipient's lifetime [2]. Although the acute rejection rate is less than 10% in the first year after transplantation [3], graft survival has not commensurately improved [3], [4]. Better monitoring methods that reflect graft status to enable individualized intervention before irreversible damage could improve long-term allograft survival [5].

Renal biopsy remains the gold standard to assess graft health, but it is controversial due to its cost and associated risk for morbidity [2]. Moreover, biopsy provides only a highly localized sample of what is known to be generally heterogeneous disease pathology [6], [7]. Rather than biopsy, noninvasive monitoring methods that identify allograft inflammation and/or fibrosis in early degenerative stages could enable timely interventions to prolong graft survival.

It has been shown that inflammation and fibrosis change the mechanical properties of renal parenchyma, and ultrasound-based methods like compression elastography [8]–[10], transient elastography [11], [12], and Acoustic Radiation Force (ARF)-induced shear wave velocity [13]–[15] have been applied to determine the changes in mechanical properties associated with renal inflammation and fibrosis. Recently, Hossain *et al.* showed that the regional ratios of VisR parameters statistically differentiated patients with allografts with chronic allograft nephropathy, glomerulonephritis, moderate vascular disease, and mild and moderate tubular/interstitial scarring from non-biopsied control allografts [16], [17]. Further, exploiting the fact that the kidney is mechanical anisotropic, i.e., mechanical properties vary with orientation [18]–[20], Hossain *et al.* showed that VisR derived the mechanical degree of anisotropy (DoA) changes with renal inflammation in a pig model [21].

These initial preclinical and clinical results suggest that VisR could improve the selectivity of renal transplant patients in need of biopsy to confirm graft disease from those for whom biopsy is an unnecessary invasive procedure with associated undue risk and cost. However, before launching a larger clinical trial to establish the diagnostic relevance of VisR renal allograft imaging, it is essential to evaluate the potential for variability in VisR outcomes due to patient demographics. It is also critical to consider the reproducibility of VisR measures by a trained practitioner. Thus, the objective of this work is to assess the variability due to donor type, sex, race, and BMI- as

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well as the reproducibility over time - of VisR-derived mechanical anisotropy measures in the cortex of healthy human renal allografts, *in vivo*.

## II. Materials and Methods

### A. Patient Population

All procedures were approved by the University of North Carolina Chapel Hill Institutional Review Board (IRB), and informed consent was obtained from all subjects. Data sets were collected under an ongoing clinical study (ClinicalTrials.gov No. NCT03079882) at the University of North Carolina (UNC) at Chapel Hill. ARFI imaging of kidney allografts was performed at two and three months after transplantation in twenty (N=20) transplant patients. All patients had stable serum creatinine levels and urine protein to creatinine ratios, so all allografts were considered healthy without clinical indication for biopsy. Note that protocol biopsies are not part of the routine standard of care for kidney transplant patients at UNC Hospitals. Patients were divided based on their sex (male: N=14 versus female: N=6), race (African American: N = 6 versus Caucasian: N=14), donor type (living: N=14 versus deceased: N =6), and BMI (BMI >30: N= 8 versus BMI < 30: N= 12).

### B. VisR Data Acquisition and Processing

VisR was performed using a Siemens Acuson Antares imaging system. VisR ensembles consisted of two reference pulses, two ARF impulses, and 69 tracking lines. The two ARF impulses were each 300-cycle (~ 71 μs) in duration. The center frequency and focal configuration of the ARF impulses were 4.21 MHz and F/1.5, respectively. The impulses were separated by 6 (0.57 ms) and followed by 75 (6.54 ms) tracking pulses. The tracking and reference pulses were conventional two-cycle A-lines at a center frequency of 6.15 MHz and pulse repetition frequency of 11.5 kHz. An F/1.5 focal configuration on transmit and dynamic focusing and aperture growth on receive (F/0.75) were used for the reference and tracking pulses. VisR ensembles (reference + ARF + tracking pulses) were acquired in forty lateral positions evenly spaced across a 2 cm lateral field of view (FOV) for two-dimensional imaging.

VisR imaging was performed as described above by an experienced sonographer. Patients were imaged on an inclined (15$^o$ from horizontal) bed in the supine position. The patients were asked to remain motionless during imaging. Imaging was performed in the superior pole of the kidney. In the longitudinal view, the lateral FOV was aligned along nephrons, such that the short axis of the ARF PSF was also aligned along nephrons. Both kidney parenchyma and a portion of the kidney sinus were visible. The imaging focal depth was selected based on the position of the sinus, generally between 3.0 and 4.0 cm. The elevational focus was set by a cylindrical lens at 3.75 cm. Then, the sonographer rotated the transducer 90$^o$ such that the short axis of the ARF PSF was aligned across the nephrons in the cortex, and ARFI data were acquired. For each transducer orientation (along and across the nephrons), three repeated acquisitions were collected to mitigate potential error from unexpected motion during data collection. Measurements over the three repeated acquisitions were averaged unless any repeated-measure contained motion artifacts, in which case that measure was removed before taking the average.

Raw RF data were saved to the hard drive of the scanner and transferred to a computational workstation for custom analysis. ARF-induced displacements were measured using one-dimensional axial normalized cross-correlation (NCC) [22]. NCC created a displacement versus time profile for each pixel. A quadratic filter [23] was applied to reduce motion artifacts before VisR ultrasound processing. The filtered displacement profiles were then fit to the MSD model using non-linear least-squares minimization. A custom C++ implementation of the Nelder-Mead algorithm was used to compute the minimization of the MSD model [24]. From the acquired VisR data, 2-D parametric images of PD, RE, and RV were generated.

### C. Anisotropy Estimation

To evaluate the degree of mechanical anisotropy using PD, RE, and RV, ROIs with dimensions 2.5 x 3.0 mm (axial x lateral) were selected. ROI positioning was performed using B-Mode guidance as follows. ROIs were positioned axially between 0.75 mm below and 1.75 mm above the imaging focal depth. Laterally, ROIs were centered in the cortex approximately 3.5 mm left of the rightmost edge of the kidney. VisR RE- and RV-based DoA was assessed by taking the ratio of these outcome metrics obtained with the short axis of the ARF PSF aligned along over across nephrons. More specifically, VisR RE- and RV-based anisotropy ratios was evaluated as ($\frac{RE_{along}}{RE_{acorss}}$), and $\frac{RV_{along}}{RV_{acorss}}$), respectively to reflect the ratio of longitudinal over transverse elastic ($\frac{\mu_L}{\mu_T}$) and viscous ($\frac{\eta_L}{\eta_T}$) moduli. However, because PD is inversely related to RE, VisR PD-based anisotropy ratio was evaluated as ($\frac{PD_{across}}{PD_{along}}$).

## III. Results and Discussion

Figure 1 shows the comparison of PD- (a), RE- (b), and RV- (c) based anisotropy ratios due to sex, race, donor type, and BMI in renal transplant patients. PD-, RE-, and RV-based anisotropy ratios were not statistically different ($p>0.05$) due to any of the examined demographic factors. The p-values comparing (PD, RE, RV) ratios between sex, race, donor types, and BMI were (0.27, 0.6, 0.54), (0.96, 0.87, 0.64), (0.59, 0.30, 0.20), and (0.62, 0.67, 0.67), respectively. The median absolute percent difference in (PD, RE, RV) ratios was (23, 13, 23) due to sex, (5, 3, 3) due to race, (0.35, 14, 13) due to donor type, and (8.6, 6, 12) due to BMI.

Bland–Altman analysis of PD-, RE-, and RV-based anisotropy ratios measured at two and three months post-transplantation were performed. The mean differences in PD-, RE-, and RV-based anisotropy ratios were -0.04, -0.11, and -0.01, respectively, suggested no meaningful bias between time

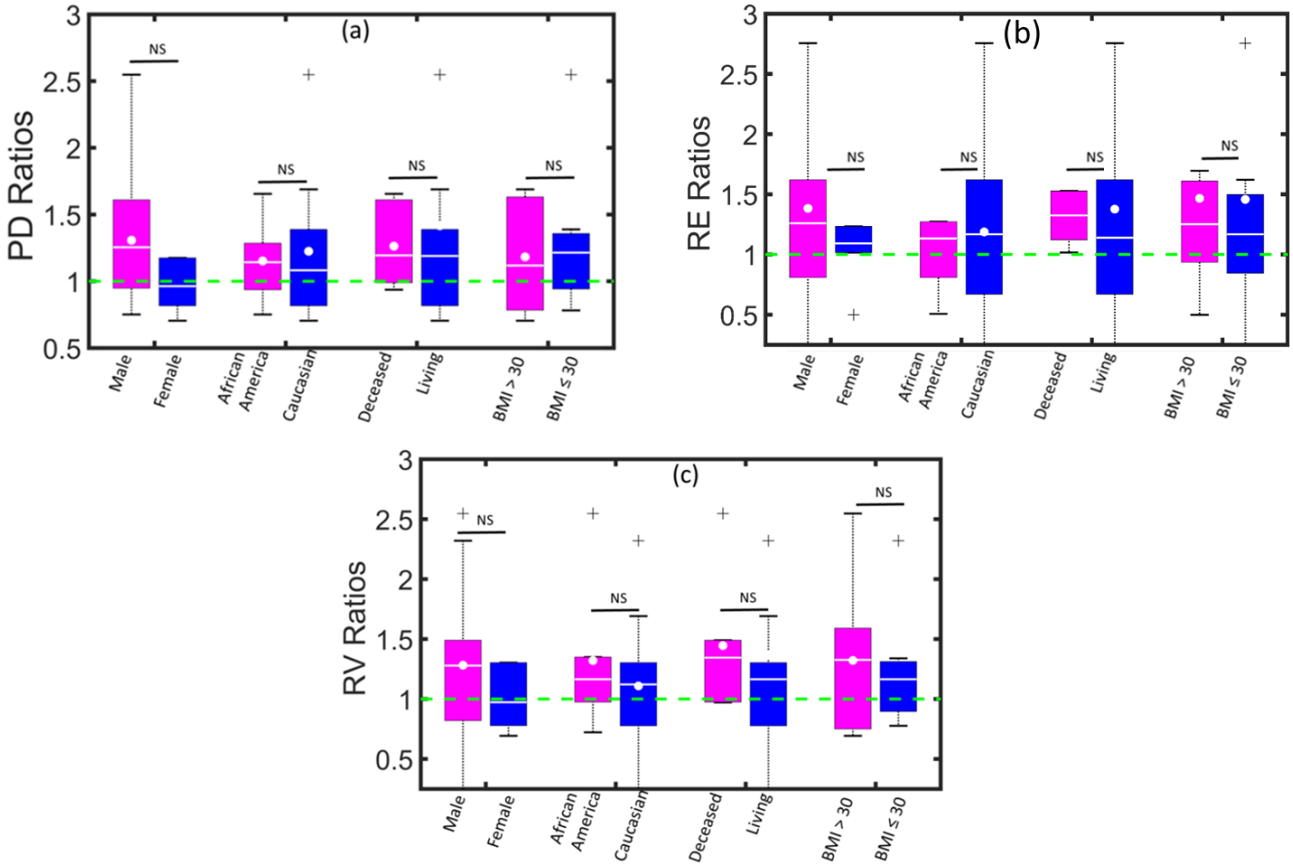


Figure 1: Comparison of PD (a), RE (b), and RV (c) ratios due to sex, race, donor type, and BMI of renal transplants patients. NS= not significant. White circle, white lines inside boxes, top box edges, and bottom box edges represent mean, median, 25th and 75th percentile values, respectively.

points. The PD-, RE- and RV-based anisotropy ratios were not statistically different from themselves acquired at two versus three months post-transplantation (p = 0.88, 0.52 and 0.91, respectively).

## IV. Conclusion

This study evaluated variability due to donor type, sex, race, and BMI as well as intra-observer reproducibility in VisR derived mechanical DoA measures in the cortex of healthy human renal allografts, *in vivo*. PD-, RE-, and RV-based anisotropy ratios were not statistically different (Wilcoxon, p>0.05) between male versus female, African American versus Caucasian, BMI > 30 versus BMI ≤ 30, and living versus deceased donor type. There was no meaningful bias in PD-, RE-, and RV-based anisotropy ratios measured two and three months post-transplantation, and these measures were not statistically different across time points. These results demonstrate that VisR derived mechanical DoA in the renal cortex is consistent across donor type, sex, race, and BMI, and reproducible by a single practitioner, in healthy kidney allografts. These findings suggest that VisR imaging is sufficiently robust for clinical investigations in kidney transplant patients, but a larger study is needed to confirm.

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