Longitudinal Comparison of Nephritic Factor Stabilizing Activity in C3 Glomerulopathy

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Background

Nephritic factors (Nef) are autoantibodies that stabilize and dysregulate the function of the C3 convertase, the cornerstone of complement amplification. Their association with the renal inflammation central to the C3 Glomerulopathies (C3G) is well reported[1,2]. It is unknown whether Nef properties 1) change over time, 2) correlate with serologic biomarker assessments, and/or 3) are useful for predicting risk for relapse/recurrence of C3G. We aimed to create a novel, concentration independent assay that allows a precise comparison of interindividual Nef stabilizing properties over various time points of disease. We further sought to correlate these results with an array of serologic biomarkers. We hypothesized that the relative degree of Nef stability would be associated with a patient-specific complement biomarker signature.

Methods

The test cohort included 6 C3G subjects who had undergone renal transplant (3 without recurrence and 3 with recurrence – see table 1 below for chronology). Biospecimens included samples before and after transplant, including at least one specimen since the time of recurrence if available. Reagent C3 convertase was formed and stabilized by injecting complement factor B, factor D, and patient-derived IgG (normal, pooled human IgG as a control) over a C3b-immobilized CM5 chip (Biacore X100) followed by injection of Decay Accelerating Factor (DAF) to remove unstabilized convertase[3]. Nef-stabilized, DAF-resistant convertases were allowed to dissociate for 3600 seconds (Fig 1). Kinetic data were collected at five time points during dissociation. Data for each clinical time point were normalized to stabilized convertase at t=0 (Fig 2). Serologic biomarker assays were performed as previously described[4].

	Diagnosis	Transplant Date	Recurrence Date	Sample 1	Sample 2	Sample 3	Sample 4
Patient 1	C3G-DDD	7/1/2019	2/27/2020	10/14/2017	10/12/2018	7/3/2019	1/6/2020
Patient 2	C3G-DDD	4/10/2017	NA	2/12/2010	7/13/2012	10/19/2016	5/23/2018
Patient 3	C3G-DDD	7/17/2019	NA	2/8/2009	12/3/2015	7/15/2019	4/10/2020
Patient 4	C3G	11/30/2018	NA	10/15/2016	11/6/2018	1/10/2019	6/16/2020
Patient 5	C3G-DDD	5/14/2019	8/2/2019	1/16/2009	11/13/2019	2/3/2020	8/3/2020
Patient 6	DDD/C3GN	7/20/2010	3/27/2012	4/14/2010	3/17/2011	3/9/2012	12/7/2012

Table 1. Patient Cohort Clinical and Sample Chronology

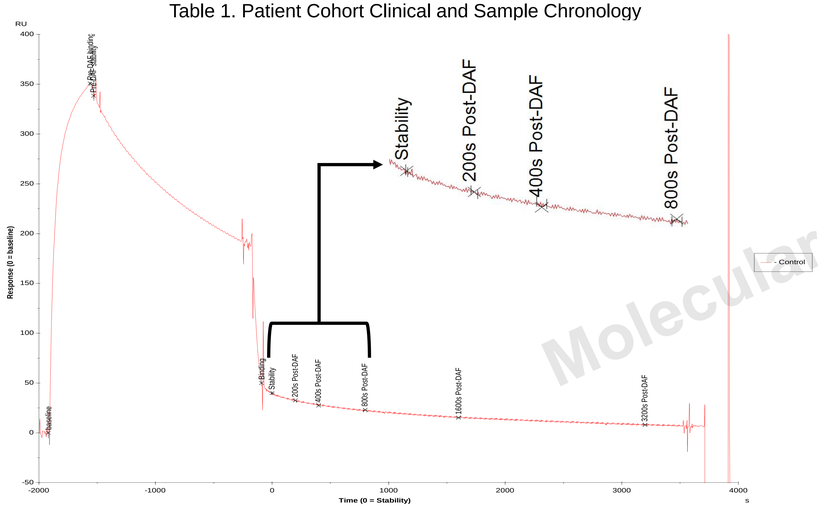

Fig 1. SPR Injection and Data Collection Scheme

Results

Nef Stabilized Convertase Decay Pattern – P1: The vertical axis represents normalized decay relative to t=0. The horizontal axis highlights six separate samples. The timepoints corresponding to the five SPR timepoints in Fig. 1 are color coded. Each box-and-whisker plot represents six replicates. At 800s, for example, patient Nef stabilizes 1.32-1.44 times more convertase than does the negative control; by 3200s, up to 2.36 times more convertase still remains. Intrasample variability at 800s was less than 13%.

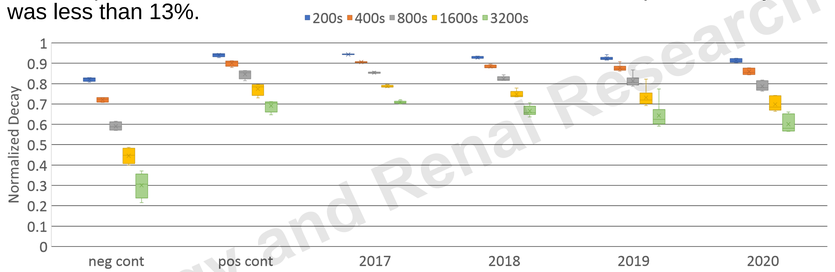

Fig 2. Patient 1 Data Analysis with Neg and Pos Controls

1. The test assay correlates with C3CSA and C3CSAP (our current Nef assays).
2. Limited complement C3 level data on 5 patients may indicate an inverse relationship between C3 level and the test Nef stability assay.

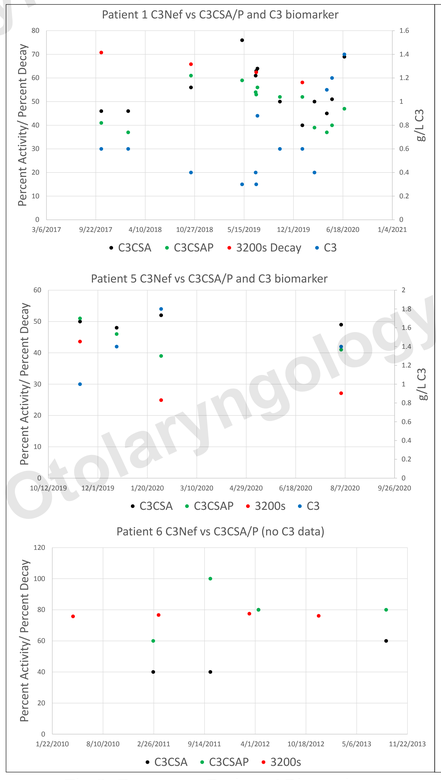

Fig 3. Recurrent Patients' Biomarkers

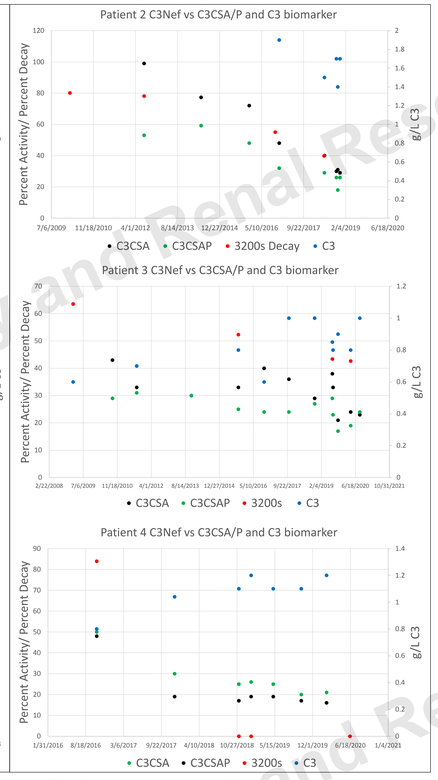

Fig 4. Non-recurrent Patients' Biomarkers

3. P2 and P3, demonstrate a consistent, moderately steep trend downward in Nef stabilizing function over time. P2: 34.8% decrease, P3: 28.3% decrease.
4. P4 Nef were DAF sensitive for specimens 2-4, therefore undetectable in our assay (significance of DAF resistance at only the first timepoint is unknown).
5. P5 had a significant decline in Nef stabilizing function between specimen 1 and 3. At last follow-up, no additional decline in stabilization function was identified.
6. P1 and P6 had minimal to no decline in Nef stabilization function over the course of disease.

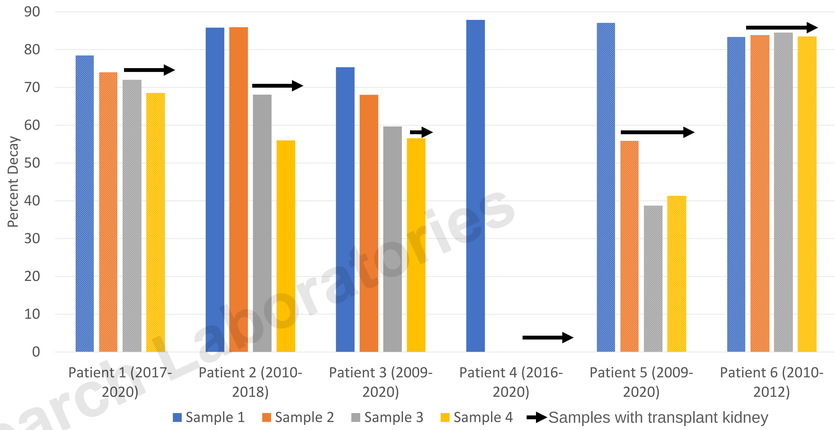

Fig 5. Median Percent Decay at 1600s for Longitudinal Patient Samples.

Conclusions

- Using a novel, concentration independent Nef assay, we provide one of the first reports describing the longitudinal stabilizing activity of Nef as a feature of C3G recurrence in transplant.
- Excluding P5, there appears to be a threshold of Nef stabilizing activity associated with recurrence of C3G.
  - Achieving a Nef stabilization activity below 60% of presentation activity appears to support a lower risk for recurrence.
- Closer review of P5 clinical and histologic history uncovers potential misclassification as C3G recurrence.

Future Directions

- Future studies will include applying this assay to additional subjects to test the hypothesis that a threshold Nef activity is associated with risk for C3G recurrence in transplant.
- Subsequent studies exchanging DAF with other complement regulators (FH, CR1) will be undertaken to further characterize Nef activity in subjects who have recurrent C3G.
- Work to identify correlations between a comprehensive array of complement biomarkers and this novel assay and how this may predict disease recurrence in transplant is ongoing.

References

1. *Nat Rev Nephrol.* 15(3), 129-143 (2019)
2. *Kidney Int.* 89, 278-288 (2016)
3. *Kidney Int.* 82, 1084–1092 (2012).
4. *Clin. J. Am. Soc. Nephrol.* 7, 265-274 (2012).

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